MRS 2014

15th International Biennial Congress of the METASTASIS RESEARCH SOCIETY

Heidelberg, Germany
June 28th - July 1st, 2014

http://metastasis-research.org/
Local Congress Organiser:
Jonathan Sleeman, PhD
President-elect of the Metastasis Research Society,
Professor of Microvascular Biology and Pathology
Medical Faculty Mannheim
University of Heidelberg, Heidelberg, Germany

Programme Committee:
Robin Anderson, Sue Eccles, Janine Erler, Kazuyuki Itoh, Yibin Kang,
David Lyden, Conor Lynch, Andrea Mastro, Klaus Pantel, Lun-Xiu Qin,
Carrie Rinker-Schaeffer, Lalita Shevde-Samant, Jonathan Sleeman, Rik
Thompson, Dan Welch, Dihua Yu and Qinghua Zhou.

We kindly thank our financial supporters:

[Logos of sponsors]
Dear Conference Participants,

Welcome to the 15th biennial conference of the Metastasis Research Society (MRS)! It is a great pleasure to be able to host you in Heidelberg. We are very grateful to the German Cancer Research Centre (DKFZ) and to Prof. Otmar Wiestler for providing the venue for the meeting, and especially to the German Research Foundation (DFG) for the substantial grant they have given in support of the conference. The MRS is also very thankful to the Association for International Cancer Research (AICR) and to the European Association for Cancer Research (EACR) for their generous financial contribution, as well as for the highly valued financial support given by the companies listed above. Please take some time during the meeting to visit their stands.

The MRS considers fostering the work and careers of young investigators in metastasis research as one of its major objectives. Over the last few years the society has undertaken a number of initiatives in this direction. The establishment of the Early Career Ambassadors of the MRS (ECAM) has been instrumental in these efforts (http://metastasis-research.org/basic-page/early-career-ambassadors-mrs). In particular we are very grateful to the Early Career Leadership Council (ECLC) of ECAM who have organized both the special ECAM session at the main conference, as well as the ECAM satellite meeting for young investigators that took place prior to the main MRS conference. Special thanks are due to Lalita Shevde-Samant and Sandra Klusmeier for their efforts. I would like to take this opportunity to draw the attention of all young investigators attending the conference to the “Meet the Professor” session on Sunday. We hope this session will provide a forum in which you can draw on the experience of established investigators to answer your questions regarding career issues and the like.

In addition to the Josh Fidler Innovation Award (kindly sponsored by Anticancer, Inc.), we are pleased this year to be able to award for the first time a new prize – the Kurt Hellmann Award Lecture – given in honour of Kurt Hellmann, one of the founding fathers of the MRS and pioneer of the therapeutic use of razoxane, who died recently at the age of 91. We are very grateful to Novartis for generously funding this prize and to Dr. Rudolf Steiner for his efforts in supporting the creation of this award. To find out more about the life and work of Kurt Hellmann, please visit the MRS web site (http://metastasis-research.org/news/memoriam-professor-kurt-hellmann).

The biennial MRS conference marks the changeover of MRS board members. Sincere thanks are due to the outgoing board members for their efforts on behalf of the society over the last years, to the outgoing MRS President Carrie Rinker-Schaeffer, and especially to the outgoing MRS Treasurer Dan Welch for his many years of service to the society. Please take a few moments to visit the new MRS web site (http://metastasis-research.org/) and consider membership if you have not already done so. We look forward to the continued development of the MRS in the coming years, and encourage you to let us know how the society can best support you and our research field.

I wish you all a stimulating and informative conference.

Best regards,

Jonathan Sleeman
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Conference Programme

Saturday 28th June

11:00 – 13:00  MRS Executive Board Meeting
DKFZ, Communication Centre

12:00  Registration desk opens

14:00 – 14:30  Welcome and Introduction
Carrie Rinker-Schaeffer and Jonathan Sleeman

14:30 – 16:30

Session 1: Clinical perspectives
Chair: Dihua Yu and Jonathan Sleeman

<table>
<thead>
<tr>
<th>Speaker</th>
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<tbody>
<tr>
<td>Peter Dubsky</td>
<td>Can bone drugs change the outcome of cancer? A clinical perspective</td>
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<td>from breast cancer</td>
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<td>Nadia Harbeck</td>
<td>uPA system in breast cancer: clinical validation as an ASCO</td>
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<td>recommended biomarker and a therapy target</td>
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<td>Brunilde Gril</td>
<td>Molecular and Preclinical Insights in Brain Metastasis of Breast</td>
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<td>Cancer</td>
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<tr>
<td>Short talk</td>
<td>Dihua Yu: Targeting driver kinases as novel therapies for breast</td>
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<td>cancer brain metastases (Abstract #B83)</td>
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<tr>
<td>Short talk</td>
<td>CJ (Dian) Corneliussen-James: What metastatic breast cancer patients</td>
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<td>want, need, know and don’t know (Abstract #A23)</td>
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16:30  Break

17:00 – 19:00

Session 2: The „Omics“ of metastasis and systems medicine
Chair: Rik Thompson

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<tr>
<th>Speaker</th>
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<tr>
<td>Christine Iacobuzio-</td>
<td>Dynamics of Clonal Progression in Pancreatic Cancer</td>
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<td>Donahue</td>
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<td>Rune Linding</td>
<td>Targeting colorectal cancer metastasis using global, quantitative</td>
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<td>and integrative network biology</td>
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<td>Birgit Schoebel</td>
<td>Using Systems Medicine to understand the impact of the variability</td>
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<td>of liposomal drug delivery to diverse tumor lesions in patients</td>
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<td>Short talk</td>
<td>Kent Hunter: Integrating SNPs, epigenetics and transcriptomics to</td>
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<td>better understand the inherited predisposition to breast cancer</td>
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<td>metastasis (Abstract #A61)</td>
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<td>Short talk</td>
<td>Eran Andrechek: A mouse model gene expression database reveals E2Fs</td>
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<td>as key regulators of breast cancer metastasis (Abstract #A9)</td>
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19:00  Welcome Buffet, DKFZ Communication Centre Foyer
Sunday 29th June

08:30 – 10:30

Session 3: Regulation of metastasis by the immune system
Chair: Andrea Mastro

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<tr>
<th>Speaker</th>
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<tbody>
<tr>
<td>Lisa Coussens</td>
<td>Inflammation and Cancer: Immune cells as targets for anti-cancer therapy</td>
</tr>
<tr>
<td>Jeffrey Pollard</td>
<td>Macrophages Promote Tumor Progression and Metastasis</td>
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<tr>
<td>Tomoharu Miyashita</td>
<td>JAMR-sponsored plenary talk: The metastasis-promoting roles of extravasated platelet aggregation in pancreatic cancer and stroma</td>
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<tr>
<td>Short talk</td>
<td>Seth Coffelt: Gamma delta T cells and neutrophils conspire together to promote breast cancer metastasis (Abstract #A20)</td>
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<tr>
<td>Short talk</td>
<td>Noona Ambartsumian: Anti-S100A4 antibody suppresses tumor progression and metastasis by blocking the recruitment of T cells to the growing tumor and the pre-metastatic niche (Abstract #A7)</td>
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10:30 Break

11:00 – 12:30

Session 4: Regulation of metastasis by inflammation
Chair: Barbara Fingleton

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<tr>
<th>Speaker</th>
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<tbody>
<tr>
<td>Fran Balkwill</td>
<td>Targeting inflammatory cytokines in cancer</td>
</tr>
<tr>
<td>Neta Erez</td>
<td>Co-evolution of Cancer-Associated Fibroblasts During Cancer Progression and Metastasis</td>
</tr>
<tr>
<td>Short talk</td>
<td>Agnieszka Swierzczak: The promotion of breast cancer metastasis caused by inhibition of CSF-1R/CSF-1 signaling is blocked by targeting the G-CSF receptor (Abstract #B60)</td>
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<tr>
<td>Short talk</td>
<td>Tobias Bald: UVB-induced neutrophilic inflammation promotes melanoma-endothelial cell interactions and metastasis (Abstract #A11)</td>
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12:30 – 13:30 Lunch

12:45 – 13:30 Meet the Professors

Room to be announced: Dan Welch, Rik Thompson, Dihua Yu
Room to be announced: Yibin Kang, Robin Anderson, Andrea Mastro

13:30 – 14:30 CEM Editorial board meeting

13:30 – 15:30 Poster session I
15:30 – 17:30

**Session 5: Therapy-stimulated metastasis**  
*Chair: Robin Anderson*

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<thead>
<tr>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>Yuval Shaked</td>
<td>The role of macrophages in tumor growth and metastasis following treatment with chemotherapy</td>
</tr>
<tr>
<td>Russell Hughes</td>
<td>A distinct subset of perivascular macrophages drive tumour relapse after chemotherapy</td>
</tr>
<tr>
<td>Hellmut Augustin</td>
<td>Angiocrine regulation of tumor metastasis: A new window of opportunity for therapeutic intervention</td>
</tr>
<tr>
<td><strong>Short talk</strong></td>
<td><strong>Natacha Leroi:</strong> Does neoadjuvant radiotherapy and the timing of surgery modify metastatic dissemination? (Abstract #A80)</td>
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<tr>
<td><strong>Short talk</strong></td>
<td><strong>Michael Lizardo:</strong> Metastatic osteosarcoma cell adaptation to proteotoxic stress in lung microenvironment involves upregulation of the endoplasmic reticulum chaperone glucose-related protein 78 (Abstract #A86)</td>
</tr>
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17:30 Break

18:00 – 19:00

*Kurt Hellmann Award Lecture*

Laudation: Dan Welch

**Sue Eccles**  
*Targeting metastatic disease: current progress and key challenges*

*Kindly sponsored by Novartis*
Monday 30th June

08:30 – 10:00

Session 6: Microenvironment and niche
Chair: Carrie Rinker-Schaeffer

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<tr>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>David Lyden</td>
<td>Tumor-derived exosomes initiate pre-metastatic niche formation and organotropism</td>
</tr>
<tr>
<td>Zena Werb</td>
<td>Regulating the tumor microenvironment in primary tumors and metastases</td>
</tr>
<tr>
<td>Short talk</td>
<td>Lalita Shevde-Saman: Cellular crosstalk mediated by Hedgehog signaling in the tumor microenvironment (Abstract #B53)</td>
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<td>Short talk</td>
<td>Anette Høyø: LOX and TEM8, a new relationship affecting tumor and metastatic growth? (Abstract #A59)</td>
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10:00 Break

10:30 – 12:00

Session 7: Circulating tumor cells
Chair: Allison Allan

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<tr>
<th>Speaker</th>
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<tbody>
<tr>
<td>Klaus Pantel</td>
<td>Circulating tumor cells: Biology and clinical implications</td>
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<tr>
<td>Andreas Trumpp</td>
<td>Circulating Metastasis-initiating Cells in Breast Cancer</td>
</tr>
<tr>
<td>Short talk</td>
<td>Miodrag Guzvic: Targeted expression profiling of single disseminated cancer cells isolated from bone marrow of prostate cancer patients (Abstract #A39)</td>
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<tr>
<td>Short talk</td>
<td>Robin Anderson: Mobilisation of viable tumor cells into circulation during radiotherapy of NSCLC (Abstract #A8)</td>
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12:00 – 12:15 Group photograph

12:15 – 13:30 Lunch

12:30 – 13:30 Workshop (main auditorium)

Dan Welch, Jim Talmadge and Roberto Buccione
A view from the Editor’s chair: tips to writing and reviewing manuscripts

Round table discussion together with Gemma Alderton

13:30 – 15:30 Poster session 2
15:30 – 17:30

**Session 8: Dormancy**  
*Chair: Klaus Pantel*

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>Julio Aguirre-Ghiso</td>
<td>Mechanisms of disseminated tumor cell dormancy: an awakening cancer biology</td>
</tr>
<tr>
<td>Curzio Rüegg</td>
<td>Mechanisms of therapy-induced metastasis and dormancy</td>
</tr>
<tr>
<td>Carrie Rinker-Schaeffer</td>
<td>Insights into the clandestine Operations of Ovarian Cancer Metastases</td>
</tr>
<tr>
<td><strong>Short talk</strong></td>
<td><strong>Andrea Mastro</strong>: Dormancy in a Dish? Growth of metastatic breast cancer cells in a bone-like microenvironment in vitro (Abstract #B8)</td>
</tr>
<tr>
<td><strong>Short talk</strong></td>
<td><strong>Veronique Orian-Rousseau</strong>: Role of CD44 in tumor progression and metastasis (Abstract #B20)</td>
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17:30 Break

18:00 – 19:00  

**Fidler Innovation Award Lecture**  
Laudation: David Lyden

**Yibin Kang**  
*How cancer cells acquire metastatic traits*

*Kindly sponsored by Anticancer, Inc.*
Tuesday 1st July

08:30 – 09:15  EACR-sponsored special imaging overview lecture
Chair: Jonathan Sleeman

Peter Friedl
Integrin-independent cancer invasion and metastatic dissemination

09:15 – 10:30

Session 9: Plasticity, cancer stem cells, EMT, therapy resistance I
Chair: Andries Zijlstra and Kazu Itoh

<table>
<thead>
<tr>
<th>Thomas Brabletz</th>
<th>EMT, microRNAs and Cancer Stem Cells</th>
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<tr>
<td>Gerhard Christofori</td>
<td>Regulatory circuits of EMT and cancer metastasis</td>
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</table>

Short talk
Radhika Nair: The Inhibitor of Differentiation proteins mediate tumor-initiating properties and metastasis in breast cancer (Abstract #B11)

Short talk
Geert Berx: Identification of a ZEB2-ZEB1-MITF transcriptional network that controls melanogenesis and melanoma progression (Abstract #A13)

10:30 Break

11:00 – 12:30

Session 10: Plasticity, cancer stem cells, EMT, therapy resistance II
Chair: Andries Zijlstra and Kazu Itoh

<table>
<thead>
<tr>
<th>Erik Thompson</th>
<th>Epithelial Mesenchymal Plasticity in Carcinoma Metastasis</th>
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<tr>
<td>Erwei Song</td>
<td>ChMRS-sponsored plenary lecture: A positive feedback loop of cancer cells undergoing EMT and tumor associated macrophages promotes breast cancer metastasis</td>
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</table>

Short talk
Rajeev Samant: Importance of regulation of NMI (N-Myc interactor) in breast cancer progression (Abstract #B45)

Short talk
Andries Zijlstra: Separation of tetraspanin CD151 from its integrin partner \(\alpha_3\beta_1\) reflects an altered migratory state and predicts prostate cancer progression (Abstract #B90)

12:30 – 14:00 Lunch

13:00 – 14:00 MRS AGM (Main Auditorium)
14:00 – 16:00

**Session 11: Early Career Ambassadors Session**  
*Chair: Lalita Shevde-Samant and Josh Neman*

<table>
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<tr>
<th>Speaker</th>
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<tbody>
<tr>
<td>Oystein Fodstad</td>
<td>Metastasis research – perspective on three decades of naivety and progress</td>
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<tr>
<td>Thomas Cox</td>
<td>Fibrosis, Cancer and the Pre-Metastatic Niche: Implications for Lysyl Oxidase</td>
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<tr>
<td><strong>Short talk</strong></td>
<td><strong>Irwin Gelman:</strong> SSεCKS/AKAP12 controls metastasis through multiple organ- and route-specific tumor/microenvironment interactions (Abstract #A37)</td>
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<tr>
<td><strong>Short talk</strong></td>
<td><strong>Vasilios Panagopoulos:</strong> Uncovering a new role for peroxidases in breast cancer development and metastasis (Abstract #B21)</td>
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<tr>
<td><strong>Short talk</strong></td>
<td><strong>Chris Madsen:</strong> STRIPAK components determine mode of cancer cell migration and metastasis (Abstract #B1)</td>
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<td><strong>Short talk</strong></td>
<td><strong>Yael Raz:</strong> Characterization of cancer associated fibroblasts in mammary gland carcinoma and lung metastasis (Abstract #B36)</td>
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16:00  Break

16:30 – 18:00

**Session 12: Metabolism and metastasis**  
*Chair: Conor Lynch*

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<th>Speaker</th>
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<tr>
<td>Dan Welch</td>
<td>Nuclear-mitochondrial cross-talk: A key determinant of cancer metastasis</td>
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<tr>
<td>Raghu Kalluri</td>
<td>MicroRNA-mediated metabolic reprogramming of breast cancer cells promotes metastasis</td>
</tr>
<tr>
<td><strong>Short talk</strong></td>
<td><strong>Jim Talmadge:</strong> Diets high in saturated fats stimulate metastasis of orthotopic mammary tumors to the brain and bone by orthotopic mammary tumors (Abstract #B61)</td>
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<td><strong>Short talk</strong></td>
<td><strong>Katherine Venmar:</strong> Elucidating the mechanisms of IL4Rα-induced mammary tumor growth (Abstract #B70)</td>
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18:00 – 18:15  **EACR- and Nature Reviews Cancer-sponsored poster awards**  
*Chairs:* Sue Eccles (EACR) and Gemma Alderton (Nature Reviews Cancer)

18:15 – 18:30  **Concluding remarks**

20:00  **Conference dinner, Hotel zum Ritter St. Georg, Heidelberg**
Speakers’ Abstracts
Saturday 28th June

Session 1: Clinical perspectives

Can bone drugs change the outcome of cancer? A clinical perspective from breast cancer

Peter Dubsky

Abstract:
uPA system in breast cancer: clinical validation as an ASCO recommended biomarker and a therapy target

Nadia Harbeck

Abstract:
Molecular and Preclinical Insights in Brain Metastasis of Breast Cancer

Brunilde Gril1, Diane Palmieri2, Yongzhen Qian3, Emily Hua1, David L. Liewehr4, Seth M. Steinberg4, Paul R. Lockman5, Quentin R. Smith6 and Patricia S. Steeg1

1. Women’s Malignancies Branch, CCR, NCI, Bethesda, MD, USA; 2. Office of the Scientific Director, NHLBI, MD, USA; 3. Frederick National Laboratory for Cancer Research, Frederick, MD, USA; 4. Biostatistics and Data Management Section, CCR, National Cancer Research, NIH, Bethesda, MD, USA; 5. Department of Basic Pharmaceutical Sciences, West Virginia University Health Sciences Center, Morgantown, WV, USA; 6. Department of Pharmaceutical Sciences, Texas Tech University HSC, Amarillo, TX, USA.

Significant advances have been made to prevent and treat early-stage breast cancer; however, metastatic breast cancer, in particular brain metastases, remains incurable. Metastasis is the main cause of mortality in cancer patients, accounting for 90% of cancer patient deaths. Brain metastases occur in up to 35% of metastatic patients whose tumors are “triple-negative” (estrogen receptor, progesterone receptor and Her2 negative) or overexpress Her2, and are expected to increase in incidence as chemotherapies improve and lead to better systemic disease control. Brain colonization of cancer cells occurs in a unique environment isolated by the blood-brain barrier (BBB), which strongly limits the penetration of drugs. Using a quantitative mouse model system for experimental breast cancer brain metastasis, vascular permeability was heterogeneous in brain metastases, with only 10% of lesions exhibiting sufficient permeability to mount an apoptotic response to taxol, suggesting that inadequate chemotherapeutic drug distribution contributes to a lack of efficacy. We have tested 18 compounds, both traditional chemotherapeutics and small molecule inhibitors, for efficacy in an experimental brain metastasis model. Partial prevention of the development of brain metastases was observed using vorinostat, lapatinib, pazopanib, TPI-287, gemcitabine and irinotecan. No drug effectively shrunk already established metastases. Our work strongly suggests that (1) an understanding of molecular underpinnings of BBB permeability in brain metastasis is crucial to improve drug delivery and develop treatment options and (2) preventive approaches for the development of brain metastases constitute a feasible clinical goal. We advocate the use of “secondary brain metastasis prevention” trials, in which patients with limited, treated brain metastases (without whole brain radiotherapy) are randomized to a preventive or placebo, with time to the development of a new metastasis as an endpoint. Close collaboration between researchers and medical oncologists will be needed to address these challenges brought on by this growing and incurable disease.
Session 2: The “Omics” of metastasis and systems medicine

Dynamics of Clonal Progression in Pancreatic Cancer

Christine A. Iacobuzio-Donahue MD PhD

Johns Hopkins Medical Institutions, The Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, GI/Liver Division, 1550 Orleans Street, CRB2, Rm 343, Baltimore MD 21231

Accumulating evidence indicates the multifactorial nature of PDA neogenesis, behavior and progression. These include both cell-autonomous and non-cell autonomous factors such as somatic genetic alterations, epigenetic modifications, immunologic suppression, metabolic dysregulation and stromal remodeling, among others. Indeed, this multitude of factors likely influence one another over the natural history of the neoplasm culminating in the lethal phenotype encountered at diagnosis. The extent to which these factors play a role throughout carcinogenesis and progression, at distinct time points or within specific contexts remains to be clarified. Moreover, the extent to which one or more factors predominate in some PDA may provide insight into subtypes of pancreatic cancer that portend improved survival, differential sensitivity to chemotherapy and radiation, or predictable dynamics of metastatic spread. This talk will center on novel data addressing the genetic evolution of PDA including evidence for the role of the microenvironment as a selective force in clonal evolution. Specifically, I will present recent data of whole genome sequencing of treatment naïve PDA and matched metastases. These data provides a framework for clonal evolution within the primary site that precedes metastasis formation, and provides the first estimation of the extent of heterogeneity among different metastases in the same distant organ. The extent to which lung and liver metastases in the same patient are clonally related to each other will also be discussed. Finally, using genetics as a method of lineage tracing of subclonal populations in the same primary tumor we show that there is stromal heterogeneity that corresponds to distinct genetic subclones suggesting a role of the microenvironment in dictating metastatic propensity.
Decoding Network Dynamics in Cancer

**Linding, Rune**

*Technical University of Denmark (DTU), Anker Engelundsvej, Building 301, Department of Systems Biology, Cellular Signal Integration Group (C-SIG), DK-2800 Lyngby, DENMARK*

Biological systems are composed of highly dynamic and interconnected molecular networks that drive biological decision processes. The goal of network biology is to describe, quantify and predict the information flow and functional behavior of living systems in a formal language and with an accuracy that parallels our characterization of other physical systems such as Jumbo-jets. Decades of targeted molecular and biological studies have led to numerous pathway models of developmental and disease related processes. However, so far no global models have been derived from pathways, capable of predicting cellular trajectories in time, space or disease. The development of high-throughput methodologies has further enhanced our ability to obtain quantitative genomic, proteomic and phenotypic readouts for many genes/proteins simultaneously. Here, I will discuss how it is now possible to derive network models through computational integration of systematic, large-scale, high-dimensional quantitative data sets. I will review our latest advances in methods for exploring phosphorylation networks. In particular I will discuss how the combination of quantitative mass-spectrometry, systems-genetics and computational algorithms (NetworKIN [1], NetPhorest [4] and KinomeXplorer [10]) made it possible for us to derive systems-level models of JNK and EphR signaling networks [2,3]. I shall discuss work we have done in comparative phospho-proteomics and network evolution[5-7]. Finally, I will discuss our most recent work in analyzing genomic sequencing data from NGS studies and how we have developed new powerful algorithms to predict the impact of disease mutations on cellular signaling networks [8,9]. I shall illustrate the power of these approaches in a recent study where we have identified colon cancer metastasis cell signaling networks.

References:

1. Linding et al., Cell 2007.
Using Systems Medicine to identify a predictive response biomarker for liposomal drugs

Birgit Schoeberl, PhD

Merrimack Pharmaceuticals, Cambridge, MA 02139, USA

At the example of MM-302, a HER2-targeted liposomal doxorubicin, and MM-398 a liposomal irinotecan, we will discuss how Systems Medicine can be used to identify a predictive response biomarker for liposomal drugs. Based on preclinical data we have built and parameterized a detailed PK/PD model that describes the biodistribution of MM-302 and free doxorubicin in the major compartments (central and peripheral compartment, tumor and individual tumor cell).

Sensitivity analysis of the PK/PD model identifies $k_{\text{trans}}$, the kinetic parameter describing the liposome extravasation from the leaky vasculature into the tumor, as rate limiting rather than the HER2 expression level of the tumor cells in order to achieve maximal levels of free doxorubicin in the tumor.

We will show preclinical data validating $k_{\text{trans}}$ as a predictive response biomarker. Based on this insight we were looking for agents to combine with MM-302 which might enhance liposome deposition. Our preclinical studies demonstrate that cyclophosphamide can significantly enhance MM-302 tumor deposition and anti-tumor activity.

Last we will show how we incorporated these preclinical insights into ongoing clinical trials with MM-398 or MM-302.
Sunday 29th June

Session 3: Regulation of metastasis by the immune system

Inflammation and Cancer: Immune cells as targets for anti-cancer therapy

Lisa M. Coussens.

Department of Cell & Developmental Biology, Knight Cancer Institute, Oregon Health & Sciences University, 3181 SW Sam Jackson Park Road, Portland OR 97239-3098. Email: coussenl@ohsu.edu

The concept that leukocytes are components of solid tumors is not new; however, their functional involvement as promoting forces in tumor progression has only recently been appreciated. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue, subsequent regulation those leukocytes exert on evolving cancer cells, and how malignant cells in turn respond to cytotoxic therapies. By studying transgenic mouse models of skin, lung, breast and pancreas cancer development, we now appreciate that adaptive leukocytes differentially regulate myeloid cell recruitment, activation, and behavior, by organ-dependent mechanisms. In turn, selective myeloid cell types provide key survival factors to malignant cells, foster angiogenic programs and invariably blunt CD8\(^+\) T cell-mediated killing of tumor cells. Treatment of transgenic mice predisposed to these various cancer types with agents that selectively block lymphocyte/myeloid-based programs results in slowing of primary tumor growth, improved responses to chemotherapeutic drugs, and significantly diminished presence of metastatic disease. To be presented will be our recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells, and new studies evaluating how attenuating protumor properties of select lymphoid and myeloid cells can be exploited to enhance therapeutic responses to cytotoxic therapy.

LMC acknowledges generous support from the NIH / NCI, the Department of Defense Era of Hope Scholar Expansion Award, Susan G. Komen Foundation, the Breast Cancer Research Foundation, and a SU2C award supported by the AACR and Lustgarten Foundation.
Macrophages Promote Tumor Progression and Metastasis.

Bin-Zhi Qian, Takanori Kitamura and Jeffrey W. Pollard

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There is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression [1]. Macrophages enhance malignancy at the primary site by stimulating angiogenesis, inducing tumor cell migration, invasion and intravasation and by suppressing anti-tumor immunity [2,3]. At metastatic sites macrophages promote tumor cell extravasation, survival and subsequent growth. Each of these activities is stimulated by a different population of macrophages whose unique signaling pathways might represent new therapeutic targets [2].

At the metastatic site Ly6c positive inflammatory monocytes (IM) are recruited to tumor cells arrested through the formation of micro-clots in the lung. This IM recruitment is caused by CCL2 expressed from tumor cells signaling via the CCR2 receptor expressed on the monocytes. These monocytes subsequently differentiate into metastasis associated macrophages (MAMs). The monocytes and MAMs stimulate tumor cell extravasation via a VEGF mediated mechanism and subsequent survival of the tumor cells in the lung. Thereafter the MAMS promote the persistent growth of metastatic lesions. Consequently inhibition of monocyte recruitment or depletion of MAMs inhibits metastasis in a variety of mouse and human models [4]. In this talk I will discuss the mechanisms behind this macrophage promotion of metastasis.

The metastasis-promoting roles of extravasated platelet aggregation in pancreatic cancer and stroma

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Background: The last decade has focused attention on the central role of platelets interacting with tumor cells and the immune system in promoting tumor progression and distant spread through release of growth factors, such as transforming growth factor beta (TGF-\(\beta\)), vascular endothelial growth factor A (VEGF-A) and plasminogen activator inhibitor-1 (PAI-1), into the tumor microenvironment. We focused on the potential metastasis-promoting role of extravasated platelet aggregation (EPA) in pancreatic cancer and stroma.

Materials and Methods: Resected pancreatic cancer specimens from 40 patients were used in this study. To examine the expression and localization of platelet aggregation in the epithelial-mesenchymal transition (EMT) region in cancer and stroma, CD42b, Snail1 and E-cadherin were assessed using immunohistochemistry. We determined the correlation of these expressed proteins with clinical features and overall survival.

Results: CD42b expression was detected at the invasive front of the tumor, which was in 73\% of the EMT portion, but not in the region of tubular formation. Increased Snail1 and loss of E-cadherin expression were noted in 85\% and 75\% of the EMT portion, respectively. There was a significant correlation between CD42b and Snail1 expression (\(p=0.02\)) and CD42b and loss of E-cadherin expression (\(p=0.008\)). No prognostic impact of CD42b, Snail1 or loss of E-cadherin expression on overall survival was identified using Kaplan–Meier survival analysis.

Conclusion: We demonstrate that EPA is associated with the first step in the formation of the EMT. These data suggest a potential role for antiplatelet agents to suppress EMT and metastasis by changing the tumor microenvironment.
Session 4: Regulation of metastasis by inflammation

Targeting Inflammatory Cytokines in Cancer

Frances Balkwill

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Cancers are driven by complex networks of cytokines and chemokines that enable communication between the malignant cells and the many other cell types of the tumor microenvironment.

Pre-clinical experiments have shown that targeting these networks with therapeutic antibodies or small molecule inhibitors can decrease tumor growth and spread. The challenge now is to translate these promising results to clinical trial. This talk will focus on the cytokine IL-6 and the chemokine receptor CCR4.

IL-6 is a key regulator of an inflammatory cytokine network of high-grade serous ovarian cancer, HGSC. Clinical, pre-clinical and in silico experiments showed that antibodies to IL-6 can have multiple actions within the tumor microenvironment including reductions in cytokine production, tumor angiogenesis and tumor macrophage infiltrate as well as reducing paraneoplastic thrombocytosis. We are now investigating rational combinations of anti-IL-6 antibodies with other treatments.

The chemokine receptor CCR4 is abnormally expressed on malignant cells as well as on leukocytes in human renal cell cancer, RCC. Patient plasma levels of the CCR4 ligands and their ratio reflect this abnormality and have prognostic significance. Both a small molecule CCR4 inhibitor and an anti-CCR4 antibody had anti-tumor activity in an RCC model with a novel mechanism of action on tumor-associated macrophages.
Co-evolution of Cancer-Associated Fibroblasts During Cancer Progression and Metastasis

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Cancer Associated Fibroblasts (CAFs) support tumorigenesis by stimulating angiogenesis, cancer cell proliferation, and invasion. We previously demonstrated that CAFs also orchestrate tumor-enhancing inflammation in multiple cancers, including mouse and human breast tumors. Although breast cancer is one of the major tumor types where CAFs were shown to be tumor promoting, there is no detailed analysis of the dynamic changes in CAFs characteristics and function in correlation with tumor progression and metastasis. We therefore set out to characterize the dynamic changes in CAFs during progression of mammary carcinogenesis, in a transgenic mouse model of human breast cancer. We profiled the transcriptome of fibroblasts isolated from normal mammary glands, or from defined tumorigenic stages of mammary carcinoma. Data analysis revealed distinct CAF gene expression signatures that correspond to different tumor stages, with only partial overlap between the stages, suggesting co-evolution of the microenvironment with tumorigenic progression. Additionally, we characterized the various sub-populations of fibroblastic cells during progression of mammary carcinoma and lung metastasis by morphometric analysis using several known mesenchymal markers. Co-labeling with α-SMA, a known marker for activated fibroblasts, and with PDGFRα, previously shown to be a robust marker for normal fibroblasts, revealed an increase in αSMA expression within the PDGFRα+ population, indicating that resident fibroblasts undergoing gradual activation are one origin for CAFs. Similarly, we show a gradual increase in α-SMA expression in lung metastases, indicating that fibroblast co-activation is operative at the metastatic site. Interestingly, we found another αSMA+ CAF population that was PDGFRα-. Using adaptive bone-marrow (BM) transplantations we show that a subpopulation of CAFs present in mammary tumors and in lung metastases are BM-derived, and are specifically recruited to mammary tumors. Thus, CAFs populations in primary breast tumors and in lung metastases are dynamic in their origin and gene expression, and co-evolve with tumor progression.
Session 5: Therapy-stimulated metastasis

The role of macrophages in tumor growth and metastasis following treatment with chemotherapy

Yuval Shaked

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Recent body of evidence suggests that almost any type of anti-cancer treatment including chemotherapy, radiation, surgery and targeted drugs can induce host molecular and cellular effects which, in turn, lead to tumor outgrowth and relapse despite an initial successful therapy outcome. Tumor relapse due to host effects is attributed to angiogenesis, tumor cell dissemination from the primary tumor and seeding at metastatic sites. Various bone marrow derived cells participate in this process, and many different factors are secreted from host cells in response to the therapy which then lead to tumor outgrowth. In this presentation I will discuss the potential roles of macrophages in the induction of tumor re-growth, angiogenesis and metastasis. I will show that ‘chemotherapy-educated’ macrophages can promote a variety of pro-tumorigenic and pro-metastatic effects in tumors and contribute to tumor cell dissemination from the primary tumor and metastatic spread. Macrophages from chemotherapy-treated mice secrete VEGF-C which in turn facilitates lymphangiogenesis in treated tumors, an effect which is hindered by blocking VEGF-C-VEGFR3 axis in macrophages. While this example postulates that blocking host mediated pro-tumorigenic effects following therapy improves therapy outcomes, other examples may show the opposite. For instance, the pro-inflammatory cytokine, IL1-β, is known to be highly elevated in the plasma of chemotherapy treated mice. However, blocking IL1-β in combination with chemotherapy counterintuitively induces metastatic spread by skewing macrophages from M1 and M2 phenotype. Taken together, our study suggests that macrophages may play several roles in tumors in response to chemotherapy, and can explain tumor re-growth and metastasis. Inhibiting the pro-tumorigenic and pro-metastatic effects of macrophages may sometimes improve therapy outcomes.
A distinct subset of perivascular macrophages drive tumour relapse after chemotherapy

Hughes Russell, Qian B, Keklikoglou I, Oakley O, Muthana M, De Palma M, Joyce J, Pollard J, Lewis C.E.

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Relapse after frontline therapies like chemotherapy is a major clinical problem for patients with inoperable primary and/or metastatic tumors. Various studies have shown that tumor-associated macrophages (TAMs) play an important role in driving tumor angiogenesis, immunosuppression and metastasis. Furthermore, we and others have shown that TAMs limit the efficacy of various anti-cancer therapies, and support tumour relapse after vascular damaging agents and irradiation. Here, we define a subset of chemotherapy-elicited F4/80\(^+\) MRC1\(^{+/Hi}\) TIE2\(^+\) VEGF\(^+\) CXCR4\(^{+/Hi}\) CD115\(^+\) TAMs in well-oxygenated, perivascular areas of solid tumours which promote relapse after cytotoxic agents. Pharmacological blockade of the recruitment of these cells using the CXCR4 antagonist ‘AMD3100’, and adoptive transfer experiments, revealed their important role in stimulating tumour revascularization and growth in post-chemotherapy tumours. These studies suggest that selective targeting of this perivascular TAM subset could delay or abolish the regrowth of inoperable primary and metastatic tumors in cancer patients after chemotherapy. Furthermore, the perivascular niche occupied by the therapy-elicited TAM population implicates them in the regulation of tumour cell intravasation and metastasis. Thus, their selective ablation could have added benefit in the prevention of post-therapy metastatic spread.
Angiocrine regulation of tumor metastasis: A new window of opportunity for therapeutic intervention

Hellmut G. Augustin

Vascular Biology, Medical Faculty Mannheim, Heidelberg University (CBTM), and German Cancer Research Center Heidelberg (DKFZ-ZMBH Alliance), Germany

It is increasingly recognized that the vascular endothelium does not just respond to exogenous cytokines (e.g., permeability-inducing agents, inflammation-inducing agents or angiogenesis-inducing agents). Instead, the endothelium itself serves as a rich source of cytokines that act on the cells in the microenvironment and control tissue responses. The endothelium may through this “angiocrine signaling” act as gatekeeper of organogenesis, tissue regeneration, and tissue homeostasis. We have recently shown that the antagonistic Angiopoietin/Tie ligand Angiopoietin-2 (Ang2) acts as critical regulator of endothelial cell angiocrine signaling. During partial hepatectomy-induced liver regeneration, Ang2 act as a dynamically-regulated rheostat to spatiotemporally orchestrate hepatocyte and LSEC proliferation through angiocrine- and autocrine-acting Ang2. Building on these findings, we hypothesized that Ang2 controlled angiocrine signaling may similarly regulate tumor metastasis. Indeed, the experiments revealed that endothelial Ang2 expression is induced by tumor cells in the metastatic niche. Endothelial cells thereby recruit metastasis-associated macrophages that enable the growth of seeded micrometastases. Proof-of-principle experiments will be presented exploiting such angiocrine signaling and novel mechanism-based combination therapies in the postsurgical adjuvant setting.
Kurt Hellmann Award Lecture
(sponsored by Novartis)

Targeting metastatic disease: current progress and key challenges

Suzanne A Eccles

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This is a tribute to Professor Kurt Hellmann, a true pioneer in translational metastasis research who in the late 1960’s described the first selective anti-metastatic agent in an experimental tumour model. Elegant papers followed, showing that the mechanism of action of ICRF159 (‘razoxane’) was via its effects on angiogenesis, long before ‘vascular normalisation’ became fashionable. However, in spite of preventing metastasis from colorectal cancer or sarcomas in the adjuvant setting and potentiating the effects of chemoradiotherapy, early trials of the compound had many setbacks. It took decades of dedication and persistence before a successor compound (dexrazoxane) found its place in the clinical armamentarium. Interestingly, its approval was primarily for its cardioprotective effect against anthracycline cytotoxic agents in both adults and children, but it has also shown efficacy via cytoprotective mechanisms in other indications such as experimental models of Alzheimer’s. Kurt’s story beautifully illustrates how science, serendipity and sheer slog are required for success – an object lesson to us all. It also reminds us that biology is complex and outcomes of research not always as expected.

Kurt was very much involved in the early development of the Metastasis Research Society as he was one of the founders of the EORTC Metastasis club from which it arose (with initially less than 30 members!) and also with what became its official journal ‘Clinical and Experimental Metastasis’. I will take a quick look back at the inception of the Metastasis Research Society at a meeting in London which I was privileged to organize with Kurt in 1984 - almost exactly 30 years ago. This will enable us to consider the ‘Problems and Prospects’ we saw then, the huge advances we have made in our understanding of the process of metastasis, the development of new and better experimental models, sophisticated \textit{in vitro} and \textit{in vivo} imaging modalities and the way all of these factors and more are beginning to have clinical impact.

My own research has been inspired by Kurt and many colleagues and friends in the MRS. I will briefly describe our multidisciplinary research efforts to develop inhibitors of some pivotal signaling pathways with a role in invasion, angiogenesis and metastasis. However, I will also highlight the issues that remain in understanding the driving forces behind established micrometastases at different sites (which I see as the main clinical problem) and the need for realistic experimental models in which to rigorously test our novel therapeutic approaches.
Monday 30th June

Session 6: Microenvironment and niche

Tumor-derived exosomes initiate pre-metastatic niche formation and organotropism


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Tumor-secreted factors have been recently recognized to be one of the main culprits for metastatic progression. Tumor-secreted factors such as VEGF-A, PlGF, TGFβ, TNF-a, and LOX have been shown to play active roles in the recruitment of bone marrow (BM)-derived cells to the primary tumor microenvironment and pre-metastatic niches. We have found that tumor-derived exosomes are abundantly secreted into the circulation in highly metastatic murine models and in patients with stage IV metastatic disease. Tumor-derived exosomes induce coagulation, vascular leakiness, inflammation, and extracellular matrix changes at pre-metastatic sites. Moreover, tumor-derived exosomes preferentially fuse and “educate” BM-derived progenitor cells to a pro-vasculogenic phenotype characterized by upregulation of Tie-2, VEGF-A, VEGFR2, TSP1 and ADAM10. We found that B16-F10 melanoma-derived exosomes help establish pre-metastatic niches, including the recruitment of “educated” BM cells, in specific organs destined to be involved in future metastasis whereas LLC lung cancer-derived exosomes predominant in the lung as the main organ of metastasis. These results suggest that tumor-derived exosomes may have a role in metastatic organotropism. We believe that the identification of specific exosomal proteins may partially explain the specific receptor-ligand binding of exosomes to a pre-metastatic niche defining an organotropic site for future metastasis.
Regulating the tumor microenvironment in primary tumors and metastases

Zena Werb

University of California, San Francisco

It is now well established that the local microenvironment, or niche, of cells contributes to various stages of cancer development. As a complex network of various inflammatory cells, fibroblasts, vascular cells and macromolecules with distinctive physical, biochemical, and biomechanical properties, the cellular microenvironment is highly dynamic and functionally diverse. The microenvironment is deregulated in diseases such as cancer and affects cancer progression by directly promoting cellular transformation and metastasis. We have used genetic and in vivo imaging techniques to study the evolution of the tumor microenvironment during tumor progression. The tumor cells directly modify the microenvironments of primary tumor and distant sites to be pro-metastatic by changing bone marrow hematopoiesis and recruiting activated myeloid cells. The stromal extracellular matrix and stromal cells communicate with each other and with the neoplastic cells to contribute to the aberrant tumor organ. Genetic and in vivo imaging techniques have revealed the interaction between stromal and epithelial cancer cells during tumor progression and the marked differences in different tumor microenvironments. Understanding how the composition and topography of microenvironment are maintained and how their deregulation influences cell behavior may help develop new therapeutic interventions by targeting the tumor niche.
Session 7: Circulating tumor cells

Circulating tumor cells: Biology and clinical implications

Klaus Pantel

Institute of Tumor Biology, University Cancer Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Sensitive methods have been developed to capture circulating tumor cells (CTCs) in the peripheral blood at the single cell level (Pantel et al., Nature Rev Cancer 2008; Kang & Pantel, Cancer Cell 2013). CTCs are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells such as tumor-associated DNA or microRNAs into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach/Pantel et al, Nature Rev Cancer 2011 & Nature Rev. Clin. Oncol. 2014). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers such as the actin bundling protein plastin-3 (Yokobori et al., Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, in vivo capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the “fishing” for CTCs from approx. 1.5 liters of blood within 30 minutes. CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res., 2013). Interestingly, the subset of EpCAM$^{low}$, CD44$^{high}$, CD47$^+$, c-Met$^+$ CTCs obtained from the peripheral blood of breast cancer patients might represent metastasis-initiator cells (Baccelli et al, Nature Biotech. 2013). Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient. Besides CTCs the analysis of ctDNA and circulating microRNAs may provide complementary information as “liquid biopsy” (Pantel & Alix-Panabieres, Cancer Res., 2013; Pantel et al., Nature Med. 2013; Schwarzenbach et al., Nature Rev. Clin. Oncol., 2014). This information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells (Wan, Pantel, Kang, Nature Med. 2013).
Circulating Metastasis-initiating Cells in Breast Cancer

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Metastasis is the foremost cause of cancer-related deaths. Metastatic spread is a complex process initiated by the dissemination, seeding and engraftment of malignant cells in sites distant to the primary tumor. It has been hypothesized that metastasis-initiating cells (MICs) are present within circulating-tumor-cells (CTCs) in the bloodstream of carcinoma patients. Indeed, the presence of CTCs in metastatic patients correlates with decreased overall survival in several malignancies, including breast cancer. Although these clinical data are consistent with the hypothesis that CTCs contain MICs, their existence, phenotype and activity has never been demonstrated. We present data showing that as low as 1900 CTCs were able to induce metastases in mice. Transplantation of primary patient blood derived CTCs induced metastatic growth in bones and liver, demonstrating the presence of MICs. FACS analysis of primary patient EPCAM\textsuperscript{+}-CTCs revealed heterogeneous inter-patient expression of the metastasis-promoting signaling receptors CD44, CD47 and MET. While the percentage of EPCAM\textsuperscript{+}CD44\textsuperscript{+}CD47\textsuperscript{+}MET\textsuperscript{+} CTCs in patient blood varied between 1.4 and 44\%, metastases both from the original patient and those derived experimentally from CTCs showed high levels of all three receptors. The data provide a first demonstration that EPCAM\textsuperscript{+}-CTCs express CD44, CD47 and MET and contain MICs, providing a molecular basis for the design of diagnostic tools to detect MICs and for developing rational-based approaches to target metastasis in breast cancer.
**Session 8: Dormancy**

Epithelial identity and p38α/β signaling suppress ErbB2-driven EMT and early dissemination in breast cancer.

Kathryn Harper¹, Maria Soledad Sosa¹, Alvaro Avivar Valderas¹, Hedayatollah Hosseini², Chandandaneep Nagi¹, Roger J. Davis³, David Entenberg⁴, John Condeelis¹, Eduardo F. Farias¹, Christoph Klein², and Julio A. Aguirre-Ghiso¹.

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Dissemination during pre-malignant stages of cancer is clinically and experimentally proven in breast cancer. However, the mechanisms regulating these events are unknown and the role of early-disseminated cancer cells (eDCC) in metastasis is also unclear. Here we show that MMTV-ErbB2 pre-malignant (PM) cancer cells, while non-tumorigenic are highly efficient in disseminating. Surprisingly, the vast majority of eDCC survive in a dormant state in target organs. However, after prolonged periods these eDCCs display metastasis-initiating capacity. ErbB2ₜ PM cancer cells that displayed a P-p38<sup>lo</sup>/P-ATF2<sup>lo</sup>/E-cadherin<sup>lo</sup> profile displayed markers of EMT and ErbB2<sup>hi</sup>/P-ATF2<sup>lo</sup>/E-cadherin<sup>lo</sup> cancer cells were found in human DCIS samples. ErbB2 overexpression activated a side branching-like program involving Wnt signaling, E-cadherin junction loss, and regulation of an EMT signature. This included the upregulation of canonical and non-canonical WNT ligands, FRZD7, TGF-β1 and EMT transcription factors (TWIST, SNAIL, SLUG). This signature was further upregulated upon p38 inhibition in ErbB2+ premalignant cancer cells. Interestingly, analysis of wild type and MKK3<sup>−/−</sup>/MKK6<sup>+/−</sup> mammary epithelial cells showed that a full EMT could only proceed when ErbB2 was overexpressed. Intravital high resolution imaging of transgenic MMTV-ErbB2-CFP pre-malignant lesions in situ revealed that p38α/β inhibition activates cancer cell motility, invadopodia formation and intravasation. This resulted in increased number of pre-malignant ErbB2+ circulating cancer cells in blood and eDCCs in lungs and bone marrow. We propose that p38α/β antagonizes ErbB2 signaling to block an organogenesis-encoded EMT program and dissemination. Significantly, we reveal that early dissemination is a source of predominantly dormant eDCC that might contribute to therapy evasion and metastatic relapse. Our findings may also explain the development of metastasis with occult primaries, which is observed in several cancers.
Mechanisms of therapy-induced metastasis and dormancy

Curzio Rüegg and collaborators

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Metastasis is the main cause of death for cancer patients. Radiotherapy and chemotherapy are widely used to treat cancer in adjuvant and curative-intent settings and provide survival advantages to many cancer patients by preventing relapses or by delaying progression. However, resistance to radiotherapy or chemotherapy occurs in a fraction of patients, which will eventually progress to advanced metastatic disease. Thus, it appears that radiotherapy and chemotherapy may induce two distinct tumor responses: induction of dormancy and sustained tumor control, or promotion of metastasis in case of resistance and tumor relapse. Furthermore, awaking of dormant tumor cell may be responsible for the late tumor relapses observed particularly in breast cancer.

In our laboratory we have established the breast 4T1 and MDA-MB-231 breast cancer transplantation models in BALB/c and NSG mice, respectively, and used to characterize response to radio and chemotherapy. We have previously shown that tumors locally recurring after radiotherapy have reduce angiogenesis, are more invasive and metastatic. The matricellular protein CYR61 and tumor-recruited cKit+CD11b+ cells contribute to metastatic progression in this model. Using a transgenic model we demonstrated that CYR61 promotes primary tumor growth, high-grade tumors, local invasion and increased angiogenesis. Using the MDA-MB-231 we demonstrated that CYR61 favors tumor cell extravasation to the lung, survival and metastatic outgrowth by promoting cell migration, invasion and anchorage-independent growth.

Using a model of chemotherapy-induced resistance, we have observed that selection of chemotherapy resistant tumor cells results in the generation of two distinct different phenotypes. Some cells retain growth capacities but acquire increased metastatic ability compared to the parental cells, while others shown reduced growth rate and up to 4 months tumor growth delay in vivo, depending on individual mice. Dormancy depends on an intact immune system, and CD4/CD8 cell depletion prevent cells from entering dormancy. Eventually about 50% of the dormant tumors, wake up at later time points. Importantly, though adoptive cell transfer experimemts we have identified CD11b cells as critical cells regulatign dormancy in this model.

Taken together, the data indicate that outcome after radio-chemotherapy is a complex event determined not only by the tumor cell properties but also by the host response. These results have relevant clinical and therapeutic implications.
Title:

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Abstract
How cancer cells acquire metastatic traits

Yibin Kang

Princeton University

Metastasis represents the most devastating stage of cancer progression and is responsible for most of cancer-related death. How and when cancer cells acquire metastatic traits is a topic of intense investigation and debate in the field. It has become clear that the development of metastatic capability in cancer cells is a continuous process that is shaped by the tissue of origin of the primary tumor, early oncogenic events, as well as the stresses tumor cells endure when they encounter different microenvironments and therapeutic treatments. Many genes play multiple functions during primary tumorigenesis and metastatic progression, and may represent ideal targets for therapeutic intervention. In this lecture, I will discuss some latest findings in our understanding of the origin and evolution of metastasis traits, with emphasis on the connection of metastasis genes to early events of tumor initiation.
Tuesday 1st July

Special imaging overview lecture
(EACR-sponsored special imaging overview lecture)

Tissue niches for cancer cell invasion in vivo

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Collective invasion results from multicellular migration of cancer cells that retain cell-cell junctions during migration. In models for melanoma, sarcoma and epithelial cancer, collective invasion represents a key route of invasion into non-neoplastic stroma. In monomorphic 3D invasion models in vitro, an obligate step of collective invasion is the degradation of extracellular matrix (ECM). Conversely, in vivo monitored by intravital multiphoton second and third harmonic generation microscopy, tissue microniches provide invasion-promoting tracks that enable collective migration along tracks of least resistance. As main routes, non-destructive contact-guidance is mediated by preformed multi-interface perimuscular, vascular and –neural tracks of 1D, 2D and 3D topography and multi-interface ultrastructure. Targeting of beta1/beta3 integrins induces unexpected plasticity of invasion, including collective and amoeboid single-cell dissemination, followed by enhanced micrometastasis, implicating integrin-independent dissemination as effective route to metastasis. In conclusion, collective cancer invasion is maintained by physicochemical programs that balance cell-intrinsic adhesion and mechanocoupling with encountered physical space and molecular cues.
Session 9: Plasticity, cancer stem cells, EMT, therapy resistance I

Metastasis: EMT, MicroRNAs and Cancer Stem Cells

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We have shown, that in particular tumor cells at the invasive front undergo an epithelial-mesenchymal transition (EMT) and aberrantly express EMT-associated transcriptional repressors, such as ZEB1. The amount of such cells strongly correlates with metastasis formation and poor clinical outcome. Strikingly, metastases show again a differentiated phenotype, indicating a mesenchymal-epithelial re-transition (MET) and support a regulatory role of the tumor environment in metastasis.

We described that the EMT-activator and transcriptional repressor ZEB1 is a crucial promoter of metastasis and demonstrated that ZEB1 inhibits expression of cell polarity factors and the microRNA-200 family, whose members are strong inducers of epithelial differentiation. These results indicate that ZEB1 triggers a microRNA-mediated feedback-loop, which stabilizes EMT and promotes dissemination of cancer cells. Moreover we detected that in addition ZEB1 controls Notch pathway activity and is necessary for the tumor initiating capacity of pancreatic and colorectal cancer cells. ZEB1 inhibits expression of miR-200c, miR-203 and miR-183, which cooperate to suppress expression of stem cell factors.

Notably, by suppressing these stemness-inhibiting microRNAs ZEB1 also induces a drug-resistance phenotype to cancer cells. We determined epigenetic modifications conferred by ZEB1, screened for epigenetic drug to restore expression of its silenced target genes and to subsequently overcome therapy resistance. Our data show that breaking the ZEB1 – miR-200 feedback loop is a treatment option for fatal tumors such as pancreatic cancer, and epigenetic modifications identified at ZEB1 target genes indicate a promising way how to interfere.
Regulatory circuits of EMT and cancer metastasis

Neha Tiwari¹, Vijay K. Tiwari², Dorothea Gruber¹, Anna Fantozzi¹, Nathalie Meyer-Schaller¹, Aleksander Kuzmanov¹, Dirk Schübeler², Eric van Nimwegen³, and Gerhard Christofori¹

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90% of all cancers originate from epithelial tissue. To leave the primary tumor, to invade into neighboring tissue and to seed metastasis in distant organs, tumor cells dissolve their cell-cell contacts and adjust their cell-matrix adhesion sites to a migratory and invasive mode. Such reversible phenomenon is known as epithelial-mesenchymal transition (EMT). The distinct stages of EMT are tightly regulated by transcriptional control circuits and involve activation and repression of a large number of genes that modulate the invasive behavior of cells. For example, Sox4 is indispensable for EMT and cell survival in vitro and for primary tumor growth and metastasis in vivo. Sox4 appears to act upstream of the Snail, Twist and Zeb transcriptional inducers of EMT and directly regulates the expression of Ezh2, encoding the Polycomb group histone methyltransferase that trimethylates histone 3 lysine 27 (H3K27me3) for gene repression. Ablation of Ezh2 expression prevents EMT, while forced expression of Ezh2 restores EMT in Sox4-deficient cells. Ezh2-mediated H3K27me3 marks associate with key EMT genes, representing an epigenetic EMT signature that predicts patient survival.

Klf4 is another example of a transcription factors playing a critical role in EMT. Loss and gain of function experiments demonstrate that the down-regulation of Klf4 expression is required for the induction of EMT in vitro and for metastasis in vivo. Reduced Klf4 expression correlates with shorter disease-free survival of subsets of breast cancer patients. Chromatin immunoprecipitation/deep-sequencing reveals that Klf4 acts as a transcriptional activator of epithelial genes and as a repressor of mesenchymal genes. Specifically, increased expression of Jnk1 (Mapk8) upon down-regulation of its transcriptional repressor Klf4 is required for EMT and cell migration. Other transcription factors that we have identified as critical regulators of EMT include Tead2 (the transcriptional effector of the Hippo-Warts signaling pathway), Lhx2 (directly inducing the expression of PDGF-B and thus its autocrine and paracrine functions), and Dlx2 (repressing TGFβ signaling and promoting EGF receptor signaling and thus promoting cell survival during EMT).

Many of the transcription factors critical for the regulation of EMT are well known for their functions in the homeostasis of embryonic and somatic stem cells. Consistent with this observation, we find that murine breast cancer cells that have undergone EMT exhibit several hallmarks of stem cells and more tumorigenic potential as compared to their epithelial counterparts. Notably, we find that the expression of several angiogenic factors is upregulated during an EMT and that the increased expression of VEGF-A is required for the increased tumorigenicity of invasive mesenchymal cells. We conclude that a high angiogenic potential is an intrinsic feature of tumor-initiating cells.
Epithelial Mesenchymal Plasticity in Carcinoma Metastasis

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Epithelial mesenchymal plasticity (EMP) allows carcinoma cells to adopt phenotypes across the full spectrum from proliferative, organised epithelial collectives to individual migratory mesenchymal cells. Through EMP, carcinoma cells are empowered for migration, invasion, therapy resistance, dormancy and metastasis. Using breast and bladder cancer model systems, we have demonstrated both stable states and dynamic transitions across the EMP spectrum, in association with differential malignant potential. Stable variants manifesting through epigenomic and transcriptomic differences exist in the PMC42 human breast cancer model, where the mesenchymal state facilitates tumourigenicity. In the human breast cancer MDA-MB-468 xenograft model, discrete zones of EMP are seen at both the outer, peripheral stromal interface and inner, hypoxic, necrotic interface, consistent with dynamic responses of these cells to EGF and hypoxia, respectively, \textit{in vitro}. Metastatically competent variants of the T24 TSU-Pr1 human bladder carcinoma model show an epithelial change via altered transcriptomic profiles. Insights emerging from these models will be described in relation to clinical observations in different carcinoma systems, and the therapeutic implications of EMP.

This work is supported in part by a National Breast Cancer Foundation (Australia) National Collaborative Research Program Grant for the EMPathy Breast Cancer Network.
A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis

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The close vicinity of cancer cells with epithelial-mesenchymal transition (EMT) and tumor-associated macrophages (TAMs) at invasive front of tumor suggests that these two types of cells may interact with each other. Here we show that mesenchymal-like breast cancer cells activate macrophages to a TAMs-like phenotype by GM-CSF. Reciprocally, CCL18 from TAMs induces EMT of cancer cells to form a positive feedback loop in co-culture systems and humanized mice. Inhibition of GM-CSF or CCL18 broke this loop and reduced cancer metastasis. High GM-CSF expression in breast cancer samples was associated with more CCL18+ macrophages, EMT of cancer cells, enhanced metastasis and reduced patients' survival. These findings suggest a positive feedback loop between GM-CSF and CCL18 is important in breast cancer metastasis.
Session 11: Early Career Ambassadors Session

Metastasis research – perspective on three decades of naivety and progress

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The process of metastasis represents one of the most complex biological events in human medicine, composed of many different steps, each of which involves a wide variety of molecules and signaling networks under tight regulatory control. Hence, studying mechanisms of metastasis is a big challenge, which can only be met by asking simple and sometimes naïve questions.

Our group has utilized human tumor metastasis models in nude mice and rats as tools for elucidating the metastatic process, and more specifically for studying homing and tissue preference of metastasis. Some of the resulting data opposed established concepts, and made us realize that the authorities are not always right. The technological advances during the last decades have provided great new means for investigating metastasis-associated factors at the molecular level. This has resulted in a wealth of new and important knowledge. However, with the plethora of data associating specific molecules and signaling pathways with the metastatic capacity of tumor cells, conclusions are too often drawn from experiences with single genes/proteins in one single cell line and/or in vivo model. In a clinical setting, we are still lacking information that can be useful to better predict tumor spread and explain organ specificity, dormancy, and therapy resistance of metastases.

Some of these aspects will be exemplified and discussed in the presentation, stressing the importance of a certain degree of sound skepticism towards established notions, but keeping the enthusiasm associated with the hope of new advances in metastasis research.
Fibrosis, Cancer and the Pre-Metastatic Niche: Implications for Lysyl Oxidase

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The mechanisms contributing to primary tumour development, metastatic progression, and tissue fibrosis share many commonalities including increased matrix deposition and remodelling. Lysyl oxidase (LOX) is an extracellular amine oxidase whose primary function is to post-translationally modify collagens and elastin in the extracellular matrix (ECM). Expression of LOX is clinically correlated with metastasis and poor patient survival and LOX mediated crosslinking of collagens at the primary tumour site has been implicated in cell invasion and malignant progression of multiple cancers. Concomitantly primary tumour LOX acts at distant sites of future metastasis. This remodelling at pre-metastatic sites creates niches permissive to metastasising tumour cell colonisation and growth. Moreover, there is a critical role for lysyl oxidase (LOX) in establishing a milieu within fibrosing tissues that is favourable to colonisation and growth of metastatic tumour cells. Together, this data provides an important link between extracellular matrix homeostasis, pre-metastatic niche formation, fibrosis and cancer.
**Session 12: Metabolism and metastasis**

**Nuclear-mitochondrial cross-talk: A key determinant of cancer metastasis**

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Despite the well recognized energetic requirements and stresses associated with metastasis, the relationships between metabolism, mitochondrial genetics and metastasis are still not well defined. Two recent lines of investigation point to a more intimate involvement of mtDNA than widely recognized. First, the metastasis suppressor KISS1 reverses the aerobic glycolysis (i.e., Warburg Effect) by regulating mitochondrial biogenesis. KISS1 re-expression results in higher $\text{pH}_{\text{Ex}}$ due to reduced lactate secretion concomitant with reduced glycolysis and a shift toward oxidative phosphorylation. KISS1-expressing cells have 30-50% more mitochondrial mass, which appears to be due to higher expression of PPAR co-activator 1 (PGC1α), a critical regulator of mitochondrial biogenesis. shRNA-mediated knockdown of KISS1 and PGC1α establish a pathway between these molecules, mitochondrial biogenesis and metastatic potential. Second, genetic crosses with a newly described MNX (mitochondrial-nuclear exchange) mice (Fenterman et al. (2013) Biochem J 455:157) suggest that mitochondrial polymorphisms (haplotypes) may control susceptibility to metastasis. Transgenic FVB/N-tg:MMTV-PyMT which spontaneously develop mammary tumors and lung metastasis with high penetrance were crossed with female MNX mice having the same nuclear background (FVB = wild-type) but with C57BL/6 or BALB/c mitochondrial DNA (mtDNA) backgrounds. Using this strategy, the mtDNA contributions to metastasis can be discriminated from nuclear DNA effects. Results demonstrate that tumor and metastasis incidence are not significantly different. But, metastasis size is greatly affected (smaller with C57BL/6; larger with BALB/c mtDNA, consistent with the published data using traditional breeding crosses (Lifsted et al. (1998) Intl J Cancer 77:640.)). Taken together, these data strongly support the concept that mitochondrial-nuclear cross-talk is a more significant determinant of metastasis than previously appreciated.

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Title:

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Abstract:
Poster Abstracts
Sunday 29th June

A1 Prognostic effect of metastasis-free interval is sustained for survival after metastasis in breast cancer

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Purpose: In breast cancer, disease-free interval (DFI) between curative resection of breast cancer and tumor recurrence harbors tumor biology and is different according to estrogen receptor (ER) status. Shorter DFI is known to be poor prognostic factor. However, prognostic influence of DFI on survival after first metastasis remains veiled. Here, we evaluated the effect of metastasis-free interval (MFI) on survival after metastasis in patient with metastatic breast cancer.

Methods: Between January 1991 and December 2013, we identified 346 patients undergoing tumor recurrence at distant site(s) after operation. All patients received curative resection and had MFI at least 6 months. MFI was defined as the interval time between operation and first metastasis. Overall survival time after first metastasis (OSM) was estimated and compared by the log-rank test and the Cox-regression hazard model.

Results: Median follow-up period was 60.0 % (51.4-68.6) months. Of all patients, 5 years-overall survival rate and 5 years-survival after metastasis rate were 49.9 % (47.1-52.7) and 21.5 % (18.9-24.1), respectively. Continuous value of MFI was significantly associated with survival after metastasis (unadjusted hazard ratio 0.986, 95% CI 0.981-0.991). When continuous value of MFI was transformed into categorical value (Short <2.5; Intermediate 2.5-5; Long>5), prognostic significance of MFI remained for OSM (P<0.001). In the univariate analysis for OSM, positive nodal status and negative ER were significant factors. In the multivariate model, continuous MFI was demonstrated to be a prognostic factor (adjusted HR 0.987, 95% CI 0.987-0.992) independence of LN status and ER status. Among the patients with known intrinsic subtypes (n=84), MFI significantly differed (P=0.001). MFI of triple-negative subtype was 21.9 month and shortest. In contrast, in luminal A, MFI was 42.1 month and highest.

Conclusions: We found that prognostic effect of MFI is sustained for survival after metastasis in breast cancer. Our findings suggest that MFI reflects tumor behavior and is associated with subtype.
A2 Effects of metformin on endothelial and cancer cells


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Epidemiological evidence has suggested that metformin, an anti-hyperglycemic agent commonly used in the treatment of type 2 diabetes, is a potential cancer preventive agent. Anti-angiogenesis represents a crucial mechanism in cancer prevention, a concept that we termed “angioprevention”. Since conflicting data concerning the anti-angiogenic action of metformin are emerging, we evaluated the effects exerted by metformin on endothelial and tumor cells as well as on angiogenesis using in vitro transcriptomic and secretomic array approaches and in vivo studies. We show that metformin inhibits endothelial cell ability to organize into capillary-like networks; this effect is partially dependent on the energy sensor AMPK. Gene expression and proteins profiling revealed paradoxic effects on several angiogenesis associated factors. We found induction of VEGF, COX2 and CXCR4 at the mRNA level and down-regulation of ADAMTS1. Interestingly, antibody array analysis showed essentially opposite regulation of numerous angiogenesis-associated proteins in endothelial and breast cancer cells.

We also observed that endothelial production of cytochrome p450 family member CYP1B1 was up-regulated by tumor cell supernatants, while metformin was able to block this effect by acting on AMPK. The metformin anti-angiogenic activity was exerted through inhibition of ERK1/2 activation, even in the presence of VEGF, while blocking AMPK activity was able to abrogate this effect. Metformin inhibited angiogenesis induced by VEGF in matrigel pellets in vivo and contrasted the increase in microvessel density in obese mice on a high fat diet. Further, it down-regulated the number of endothelial precursor cells from white adipose tissue in obese mice.

Our data show that metformin exerts an anti-angiogenic activity in vitro and in vivo, which is associated with a contradictory enhancement of chemokines and other inflammatory pro-angiogenic mediators, as well as a different regulation in endothelial and breast cancer cells.
A3 The pro-angiogenic phenotype and function of natural killer cell subsets: a new paradigm of inflammatory infiltrates in tumors

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Chronic inflammation is linked to the development of at least 30% of all cancers and drives carcinogenesis and tumor progression through a series of mechanisms, including induction of angiogenesis and alteration of the tumor microenvironment (TME). Tumors and tumor inflammation are able to polarize several cellular components of the immune system towards an immuno-suppressive, pro-angiogenic, pro-tumor phenotype. However, the role of natural killer (NK) cells in tumor angiogenesis remains to be defined. Several NK cell subsets have been described; among these, the major subset, approximately 95% of peripheral blood NK cells, is CD56dimCD16+ and exerts strong cytotoxic activity. Approximately 5% of peripheral blood NK cells are CD56brightCD16+ and show cytotoxicity through strong cytokine production. In the developing decidua a third NK subset, termed decidual-NKs (dNKs) has been discovered. dNKs, described as CD56superbrightCD16− display a cytokine-secreting, highly-angiogenic phenotype.

We investigated NK cell subset distribution and functions in surgically resected non-small cell lung cancer (NSCLC) and colo-rectal cancer (CRC) by flow cytometry and functional studies. NK cells infiltrating tumors and non-tumor tissues as well as peripheral blood and samples from patients without oncologic disease were used as negative controls.

The CD56dimCD16NK phenotype predominated in both all NSCLC and CRC samples while the CD56dimCD16+ cytotoxic NK phenotype prevailed in the patient peripheral blood, NSCLC adjacent normal tissues as well as in non-oncologic patient lung tissue. Further, the CD56+CD16− NK subset was associated with angiogenic cytokine production, including VEGF, PlGF and IL-8 (CXCL8) both in NSCLC and CRC samples.

Interestingly, patients with lung squamous cell carcinomas (SQK) showed significantly higher angiogenic factor production by CD56+CD16− NK cells in the resected tissues and peripheral blood, when compared to those with adenocarcinoma (ADK) or control tissues.

Moreover, supernatants derived from NSCLC, as well as from CRC infiltrating CD56+CD16− NK cells are able to induce endothelial cell capillary-like structures formation in vitro. Noteworthy this effect was particularly enhanced in SQK-NSCLC derived NK cell supernatants. In contrast supernatants of NK cells from non oncologic samples showed no angiogenic activity.

Finally, we find out that exposure of peripheral blood NK cells from healthy donors to TGFbeta1, which represents an abundant cytokine in the TME, strongly up-regulated VEGF and PlGF production, suggesting that this could represent one mechanism contributing to tumor NK cell polarization.

These data place a new player in inflammation and the immune system at the center of the pro-angiogenic state that is key for tumor development and progression.
A4 VEGFR3 plays a role in macrophage-induced lymphangiogenesis and metastasis following paclitaxel chemotherapy

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Recent studies indicate that while chemotherapy strongly restricts or reverses tumor growth, the response of the normal host tissue to the therapy can counteract the anti-tumor activity by promoting the re-growth of residual tumor cells and the outgrowth of metastases. We investigated the pro-lymphangiogenic growth factor, VEGF-C, which was highly expressed in plasma of PTX-treated mice. Plasma from PTX-treated mice induced migration, invasion, and tube formation of lymphatic endothelial cells when compared to plasma from control mice. In both 4T1 breast and Lewis lung carcinomas an increased number of lymphatic vessels positioned at the center of tumors were found in PTX-treated mice when compared to tumors from control mice, in which the lymphatic vessels positioned only at the tumor periphery. Since VEGFR3 is a major receptor for VEGF-C-induced lymphangiogenesis, we evaluated the therapeutic effect of anti-VEGFR3 blocking antibody (31C1) in combination with PTX. 4T1 bearing mice treated with PTX+31C1 exhibited a decline in primary tumor growth and inhibition of metastases when compared to mice treated with PTX monotherapy. Importantly, an abundant number of macrophages highly secreting VEGF-C colonized 4T1 tumors from PTX-treated mice when compared to tumors from untreated mice. Consequently, when 4T1 cells were co-implanted with macrophages from PTX+31C1-treated mice, primary tumor growth was delayed and fewer lung metastases were observed when compared to 4T1 cells co-implanted with macrophages from PTX-treated mice. To further elucidate the mechanism of macrophages-induced metastasis, we focused on heparanse, which is known to promote metastasis and lymphangiogenesis. While the activity of endogenous heparanse in macrophages did not change between PTX and PTX+31C1-treated mice, exogenous heparanase activity was highly elevated in macrophages from PTX-treated mice when compared to control or PTX+31C1-treated mice, suggesting that VEGFR3 blockade in macrophages may alter heparanse activity. Overall, our results suggest that the disruption of VEGF-C-VEGFR3 pathway inhibits metastasis not only by directly affecting lymphatic endothelial cells, but also by blocking macrophages’ lymphatic activity.
Inflammatory breast cancer (IBC) is the most lethal form of breast cancer because of its propensity for rapid onset of disseminated metastases, which are present in one-third of cases at diagnosis. RhoC GTPase, known to play an important role in the movement of cells, is overexpressed in 90% of IBC tumors. The mechanism that causes this increase in RhoC expression remains unknown, however, tumor associated macrophages (TAMs) have been found to facilitate the movement and invasion of many breast cancers. Therefore, as a component of the immune system attracted to sites of inflammation, we hypothesized that TAMs play a role in increasing RhoC expression in IBC cells, which consequently leads to IBC’s severe migratory and metastatic potential.

The expression of RhoC significantly increased in two different IBC cell lines, SUM149 and SUM190, in the presence of conditioned media (CM) from the macrophage-differentiated U937 monocytic cell line. This increase was not observed in either the normal-like MCF-10A breast epithelial cell line or the non-IBC MDA-MB-231 basal-like breast cancer cell line. Following upon these observations, we designed a novel microfluidic device to study the migratory phenotype of individual cancer cells in response to the macrophage CM. This device allows for the precise quantification of the migration distances of a population of cells on an individual basis, separating laggard cells from extreme migrators and uncovering the relative proportions of each. As expected, the presence of a serum gradient promoted the chemotaxis of IBC cells over a serum-free control and a CM gradient also increased the migration of IBC cells to a moderate degree. However, in the dual presence of CM without a gradient and serum with a gradient, the cells exhibited an extreme migratory phenotype and migrated roughly twice the distance of the serum-only control. Many of the lagging cells had been converted to extreme migrators. We conclude that the CM acts to “prime” the migration capability of the IBC cells in order to manifest an amplified response to the serum chemoattractant. In SUM149 stably-transfected with shRNA targeted to RhoC, we observed decreased migration when exposed to the conditioned media. Therefore, RhoC expression is necessary for this increase in migration.

Cytokine array studies have shown CCL2 and CCL5 to be key mediators in the macrophage CM and we will discuss the specific intracellular signaling pathways responsible for the increase in RhoC expression and migration capability. Studies involving RhoC inhibitors are ongoing, but could yield promising therapies for the prevention of metastasis of IBC. By understanding the specific mechanism of TAMs’ effects on IBC, we hope to learn how to control the lethal metastatic nature of inflammatory breast cancer.
A6 A systematic approach to the metastatically relevant microRNA landscape

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Metastasis is the major determinant of cancer outcome. To define the metastasis-associated microRNA landscape in colorectal cancer, we systematically analysed miRNA and mRNA expression in primary human tumors, their matched metastases and corresponding normal tissues. This allowed us to identify a miR signature that is exclusively differentially expressed in metastases, and which includes both established and novel candidate miRs such as miR-552, -218, -135, -210 and -654. Bioinformatic analysis revealed that the epithelial-mesenchymal transition (EMT) is a significant target of the metastasis-associated miR signature. Accordingly, functional studies identified three miRs as key drivers of the EMT-associated cadherin switch, as well as invasion and metastasis. These miRs act by regulating a number of novel targets, including the tumor suppressors SIAH1 and SETD2, and the transcription factor FOXN3. Together these data identify an important new miR-regulated network that fosters metastasis, validating the utility of our novel miR profiles for hypothesis generation.
A7 Anti-S100A4 Antibody Suppresses Tumor Progression and Metastasis by Blocking the Recruitment of T Cells to the Growing Tumor and the Pre-metastatic Niche.

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The tumor microenvironment plays a determinative role in stimulating tumor progression and metastasis. It is here where an intensive multi-lateral cross-talk between cancer cells and different stroma cells occurs. This cross-talk is not restricted to the site of the primary tumor but also takes place at the metastatic niche upon arrival of cancer cells. The small Ca-binding protein, S100A4, has a well-established metastasis-promoting activity. Its expression is tightly correlated with poor prognosis in patients with numerous types of cancer. Mechanistically, the extracellular S100A4 drives metastasis by affecting the tumor microenvironment. The S100A4 protein is known as an inducer of inflammatory processes and has been shown to attract T-cells to the primary tumor and to the pre-metastatic niche. In attempt to develop an S100A4-based anti-metastatic therapy we isolated a S100A4 neutralizing antibody, 6B12.

The 6B12 antibody efficiently suppressed the metastatic ability of CSML100 mouse mammary cancer cells grafted to mice. Moreover, treatment of PyMT spontaneous metastatic mammary tumor-bearing mice with this antibody led to delayed tumor growth and inhibition of metastases. Finally use of this antibody in mouse model of pre-metastatic niche formation led to the obstruction of the pre-metastatic niche. Our study revealed that the 6B12 antibody suppressed the T-cell attraction to the site of the primary tumor and to the pre-metastatic niche.

In vitro, S100A4-challenged T-cells produced an altered spectrum of cytokines implying that S100A4 is capable to induce changes in the T-cell differentiation pattern. S100A4 altered the expression of transcription factors and signal transduction pathway genes involved in the T-cell lineage differentiation. Furthermore, long-term cultivation of T-cells with S100A4 shifted the balance of T-cell polarization towards predominance of the Th2 pro-tumorigenic phenotype. The Th1/Th2 balance was restored by the 6B12 antibody.

We propose therefore that S100A4 promotes metastasis by attracting T-cells and shifting the Th1/Th2 polarization balance, thus increasing the sub-population of tumor-promoting T-cells. The 6b12 antibody blocks the attraction of T cells in vivo and rescues the T-cell differentiation pattern tilted by S100A4.

We conclude here that this antibody could serve as a solid basis for development of an efficient anti-metastatic therapy.
A8  Mobilisation of Viable Tumour Cells into Circulation During Radiotherapy of NSCLC

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Thousands of patients die annually with distant metastasis after curative-intent or “radical” radiotherapy (RT). Because non-small cell lung cancer (NSCLC), the most common cause of cancer-related mortality exhibits an especially high rate of distant metastasis after radical RT or chemo-RT for locoregionally-advanced disease, it represents a suitable model to investigate the relationship between RT and metastasis. We hypothesised that disruption of tumour architecture during RT could result in the release of viable tumour cells into the peripheral circulation.

We enumerated circulating tumour cells (CTCs) by fluorescence microscopy of blood samples immunostained with conventional CTC markers. We measured their DNA damage levels using $\gamma$-H2AX, a biomarker for radiation-induced DNA double-strand breaks, either by fluorescence-activated sorting (FACS) or by immunofluorescence microscopy. Twenty-seven RT-treated NSCLC patients had blood samples analysed by one or more methods. We identified increased CTC numbers after commencement of RT in 7 of 9 patients treated with palliative RT and in 4 of 8 patients treated with curative-intent RT. CTCs were also identified, either as single cells or in large clusters during RT by cytopathological examination (in all 5 cases studied). Elevated $\gamma$-H2AX signal in post-RT blood samples signified the presence of CTCs derived from irradiated tumours. Blood taken after the commencement of RT contained tumour cells that proliferated extensively \textit{in vitro} (in all 6 cases studied), and preliminary experiments reveal that these cells survive in NOD/SCID mice for several months, forming tumours in the lung. CTCs developed $\gamma$-H2AX foci in response to \textit{ex vivo} irradiation providing further evidence of their viability (1).

Although of concern, these findings could represent an opportunity to monitor and target CTCs during RT. They provide a rationale for the development of strategies to reduce the concentration of viable CTCs by modulating RT fractionation or by co-administering systemic therapies.

A9 A mouse model gene expression database reveals E2Fs as key regulators of breast cancer metastasis

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Metastasis is primarily responsible for the mortality associated with breast cancer. Despite this, the molecular underpinnings of cancer progression are poorly understood. By integrating bioinformatics with traditional genetics, we have addressed the hypothesis that genetic events mediating breast cancer metastasis can be elucidated from mouse models. To test this hypothesis, we assembled a gene expression database of over 1100 mouse breast cancers from 23 different models. Using this database, E2F transcription factors were predicted to be strikingly activated in metastatic mouse models. Transgenic MMTV-Neu mice were interbred with E2F transcription factor knockouts to test this hypothesis. Tumor latency was increased significantly with the loss of any of the activator E2Fs in the MMTV-Neu mice. Strikingly, there was also a significant reduction in the extent of pulmonary metastasis with loss of either E2F1 or E2F2. This metastasis reduction was shown to be a cell autonomous effect through a transplant experiment. Moreover, CD-31 staining revealed vasculature alterations with E2F loss. These data led to the hypothesis that E2F transcription factors were critical in mediating metastasis. Highly metastatic MMTV-PyMT transgenic mice also had predicted E2F transcription factor activation. Genetically testing this prediction, we noted that PyMT transgenic mice lacking E2F1 or E2F2 recapitulated the MMTV-Neu results with a vast reduction in metastasis. Using a tail vein injection we noted defects in extravasation with E2F1 or E2F2 loss. To determine the mechanism by which E2Fs regulated metastatic progression, we examined gene expression profiles of MMTV-Neu and PyMT tumors in both control and E2F knockout backgrounds. This analysis identified a cohort of genes with reduced expression in the non-metastatic E2F knockout breast cancers relative to the metastatic wild type E2F controls. Importantly, genes identified in the mouse model system are co-amplified in human HER2+ breast cancer but are not part of the HER2 amplicon. This amplicon is present in over 30% of HER2+ human breast cancers and is inversely associated with metastasis free survival. In addition to examining the amplicon genes in HER2+ human breast cancer, we have also examined predicted E2F activity using genomic signatures. This revealed four common combinations of E2F activity in HER2+ breast cancer. Importantly, low E2F1 and high E2F2 in the same sample was associated with far better relapse free survival than the opposite E2F pattern. These data indicate that the effects and the mechanisms uncovered in the mouse models are present in HER2+ human breast cancer. Taken together, by integrating bioinformatics with mouse models of breast cancer we have demonstrated that the E2F transcription factors play a pivotal role in progression from primary breast cancer to metastasis.
A10  Fibroblast activation protein increases metastatic potential but also boosts response to chemotherapy

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Fibroblast activation protein (FAP), an integral membrane serine protease, is found on most sarcoma as well as on cancer-associated fibroblasts and mesenchymal stem cells in over 90% of human epithelial cancers. In normal adult tissues, FAP is expressed only in wound healing, and is therefore an attractive target for antibody immunotherapy of cancer. We aim to clarify the role and mechanisms of action of FAP in tumour development and metastasis.

Following transfection of fibrosarcoma line HT1080 with enzymatically active and inactive FAP, FAP-expressing cells showed higher levels of adhesion to extracellular matrix proteins, as well as being more migratory and invasive, which could be blocked using an anti-FAP antibody. Interestingly, these changes were not dependent on FAP serine protease activity, but instead relied on alterations of intra- and extracellular signaling, in particular via PI3K and integrin-related signaling proteins as well as altered inflammatory cytokine secretion.

Cells expressing FAP were more susceptible to fibrosarcoma chemotherapeutic doxorubicin-induced cell death, which occurred via different cell death pathways from those that were prominent in the absence of FAP. Again, the change was not dependent on FAP’s serine protease activity. FAP-positive cells exhibited altered mitochondrial metabolism and increased levels of oxidative stress response.

In conclusion, expression of fibroblast activation protein, as found in the stroma of most epithelial-derived tumours and on sarcomas, increases tumour invasive potential and increases susceptibility to cell death caused by fibrosarcoma-directed chemotherapy. These effects are mediated via FAP’s influence on intracellular signaling pathways and cytokine secretion, rather than through its serine protease activity. Modulating FAP by targeted therapy may be a useful approach to anticancer treatment, but the implications in terms of response to treatment and changes in metastatic potential should be considered.
A11 UVB-induced neutrophilic inflammation promotes melanoma-endothelial cell interactions and metastasis

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Ultraviolet (UV) exposure represents an important etiologic factor in the development of malignant melanoma. With the advent of next generation sequencing technologies the ability of UV radiation to cause tumour-initiating DNA mutations is now clearly established. It has been hypothesised that UV irradiation also affects epidermal keratinocytes and immune cells which additionally can promote melanoma development by stimulating the survival, proliferation and migration of transformed melanocytes. How these microenvironmental effects of UV irradiation influence melanoma pathogenesis is incompletely understood. We experimentally investigated the impact of UVB-induced inflammatory responses on the progression of DMBA-induced primary melanomas in adult Hgf-Cdk4R24C mice as well as on serial melanoma transplants in syngeneic wild type and Toll like receptor (Tlr) 4 deficient mice. These model systems allowed us to study the tumor-promoting effects of UVB-irradiation independent of its tumor-initiating effects. Repetitive sun burning doses of UVB induce a neutrophilic skin inflammatory response that did not alter the incidence, multiplicity and growth kinetics of DMBA-induced primary cutaneous melanomas in a cohort of Hgf-Cdk4R24C mice compared to non-irradiated controls. However, detailed pathological analyses of UVB-irradiated melanomas revealed that the expansion of melanoma cells along the abluminal site of blood vessels in a pericyte-like location which occasionally became macroscopically visible in the dermis was significantly enhanced. This phenomenon was originally described as angiotropism by histopathologists in human melanomas. Consistent with observations in the human system, we found that enhanced angiotropism was associated with significantly increased numbers of lung metastases. Furthermore, UVB-irradiation of mice bearing serial melanoma skin transplants also enhanced angiotropism and increased the number of spontaneous metastases in the lung. Importantly, we found that the metastasis-promoting effects of UVB irradiation was abrogated in mice lacking functional Tlr4-signaling or Ly6G+ neutrophils abrogated. In summary, our work provides evidence that repetitive UVB-irradiation induces a Tlr4-dependent neutrophilic inflammation that catalyses reciprocal melanoma-endothelial cell interactions and drives the perivascular invasion of melanoma cells. A better understanding of inflammation-induced interactions between melanoma and endothelial cells may lead to new treatment approaches that impair metastatic progression of primary melanomas.
A12  AlphaVbeta3-integrin Reprograms Cancer Luminal Progenitor Like Cells to Differentiate to a Normal Like Phenotype

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Loss of cell polarization and luminal filling of mammary glands are key structural alterations in breast cancer disease. Recent findings support the notion that disruption of cell-polarity mechanisms plays a causal role in tumor initiation, thus implicating a role of cell and tissue polarity mechanisms as potential non-canonical tumor suppressors. We present here for the first time the role of beta 3-integrin (Intβ3) in promoting the commitment of luminal breast cancer progenitor like cells to differentiate to polarized acini like structure. Our results demonstrate that overexpression of Intβ3, in MCF-7 (MCF-7-Intβ3) and T47D cells (T47D-Intβ3) promote their reversion to acini like organoids resembling a normal breast tissue, when cultured in the physiological relevant 3 dimensional basement membrane extract (3D BME system). The phenotypic reversion was demonstrated by the generation of organoids that gradually progressed into spherical lumen containing structures with apicobasal polarity displayed by the apical expression of mucin-1 (MUC1), and basal expression of laminin 5. These acini like structures resembled normal alveolar cells displayed also by their expression of the milk protein beta casein. Conversely, control MCF-7 and T47D cells stably expressing empty vector (MCF-7-vec, T47D-vec respectively) did not undergo phenotypic reversion. Intriguingly, this reversion was driven by cancer luminal progenitor like cells derived from non-adherent mammospheres (either expressing CD44<sup>-/low</sup> CD24<sup>high</sup> or EpCAM<sup>high</sup>CD49f<sup>low</sup> phenotype) that expressed integrin αVβ3. Whereas, integrin αVβ3<sup>-neg</sup> cancer luminal progenitor like cells could not differentiate to acini like structure in the 3D BME system. Furthermore, inhibiting integrin αVβ3 activation in cancer luminal progenitor like cells with Cilengitide inhibited their differentiation. Therefore, these results suggest that commitment of the cancer luminal progenitor like cells to differentiate is dependent on integrin αVβ3 expression and activation. Furthermore, we demonstrated that the reversion of MCF-7-Intβ3 cells to a normal like phenotype was mediated by downregulation of Notch4 expression and downstream signaling and can be partially reversed by inhibiting αVβ3 activation. Importantly, the reversion of MCF-7-Intβ3 cells to a normal like phenotype induced a dormant state depicted by induction of p21 expression and reduction in Ki67 positive cells compared to MCF-7-vec cells. Hence, these findings propose a novel strategy to normalize the malignant phenotype by reprogramming cancer luminal progenitor like cells to differentiate via the expression of integrin αVβ3. Therefore, promoting such differentiation of luminal breast cancer cells in conjunction to their microenvironment may serve as a novel approach to combat local recurrences of breast cancers that resist conventional therapies, thus keeping them on halt.
A13 Identification of a ZEB2-ZEB1-MITF transcriptional network that controls melanogenesis and melanoma progression

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The ZEB family of transcription factors consists of two members, ZEB1 (deltaEF1) and ZEB2. The ZEB proteins are crucial for migration of neural crest cells and formation of their derivative structures during subsequent embryonic development. Detachment of single cells and invasion into the surrounding tissue are necessary for these processes which are also considered to be the driving forces in malignant tumor progression. To investigate the importance of ZEB2 during both melanocyte homeostasis and melanoma progression, we used the melanocyte specific Tyr-Cre mouse line to conditionally delete or overexpress ZEB2 in the melanocyte lineage. Our data clearly show that ZEB2 plays a crucial role in melanogenesis as melanocyte specific deletion of ZEB2 (ZEB2\textsuperscript{MC-KO}) causes congenital loss of hair pigmentation. Interestingly, although melanoblast migration was affected by the absence of ZEB2, a fraction of melanocytes were still able to reach the bulge and bulb area of the hair follicles, where they remain undifferentiated. Immunohistochemical data further demonstrate that several genes of the melanocyte differentiation pathway, such as the master regulator of melanogenesis MITF are strongly reduced in ZEB2\textsuperscript{MC-KO} hair follicles. Importantly, by analyzing ZEB2 protein expression in human primary melanoma and metastases tissues, we found that ZEB2 is present at high levels in several of these clinical specimens. In order to assess the contribution of ZEB2 in melanoma, we used the Tyr::N-Ras\textsuperscript{Q61K} preclinical mouse melanoma model in which we modulated ZEB2 expression in the melanocytes. Our results clearly indicate that the status of ZEB2 is strongly related with the formation of highly malignant melanoma which metastasizes to the lymph nodes, liver, lungs and the peritoneum. Our data are also relevant for human melanomagenesis as the ZEB2 expression status is associated with patient survival. In conclusion, we demonstrate that ZEB2 has a crucial role in the differentiation status and migratory behavior of both melanocytes and melanoma cells.
Metastasis is the primary cause of cancer-related mortality and and the metastatic microenvironment consisting of leukocytes and stromal cells significantly affect cancer progression. Cross-talk between tumor cells and their microenvironment is facilitated by a variety of soluble factors, including growth factors, cytokines such as chemokines. Elevated expression of the chemokine CCL2 correlates with enhanced metastasis and thereby poor prognosis in e.g. breast, colon, and prostate cancers. Chemokines affect various processes including leukocyte recruitment, angiogenesis, tumor cell survival, tumor cell adhesion, proliferation, vascular permeability, immune suppression, and metastasis. In particular, inflammatory chemokines CCL2 and CCL5 are the main mediators of inflammatory monocyte CCR2^Ly6C^high recruitment to metastatic sites. Accordingly, expression of both chemokines correlated with enhanced tumor cell extravasation and metastasis in experimental models. Recently, we described a novel mechanism of tumor cell extravasation that is dependent on endothelial-CCR2 expression. Using both in vivo and in vitro approaches we demonstrated that colon carcinoma-derived CCL2 activates CCR2 on endothelial cells, which induces vascular permeability, thereby enabling efficient tumor cell extravasation. Of note, the process of tumor cell extravasation was significantly promoted by inflammatory monocytes. Interestingly, induction of vascular permeability through endothelial CCR2 signaling is dependent on JAK2-Stat5 and p38MAPK signaling. Preliminary results indicate that CCL2 and CCL5 exert different functions during metastatic colonization. Our current study delineates the mechanisms enabling tumor cell extravasation and the role of myeloid-derived cells in this process.
Breast cancer cell quiescence in bone is orchestrated by osteoblasts transitioned into Tumor-Associated Fibroblasts via exosomal miRs

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Breast cancer frequently metastasizes to bone. Upon entry to a secondary site, disseminated breast cancer cells (BCCs) can undergo a period of proliferative quiescence for ≤25 years, where they remain clinically undetectable and untreatable. Despite the high prevalence of dormant tumors in humans, this area of research is poorly understood.

We propose that disseminated BCCs educate osteoblasts (OBs) into a tumor-associated fibroblast (TAF)-like cell that, in turn, orchestrates the proliferative quiescence BCC phenotype in bone. To test this, differentiated MC3T3-E1 murine osteoblasts (OBs) were incubated for 21 days with conditioned medium (CM) from metastatic BCC variants (MDA-231 or metastasis-suppressed MDA-231). OB cell lysates were analyzed for TAF proteins. Importantly, OBs reduced their expression of alkaline phosphatase, F4/80, CD31, MMP9, and alpha SMA, with the largest change seen in 41-day-old conditioned OBs. These results are consistent with the described aggressive “matrix-remodeling” TAF. On the other hand, metastasis-suppressed BC CM treated OBs increased their expression of IL-6, suggestive of an OB inflammatory response to disseminated BCCs.

As a way to better understand how TAF-OBs can induce proliferative quiescence in BCC we sought to examine the roles of gap junctions and exchange of miRs as modulators to this process. Briefly, OBs were early or late differentiated (10 or 20 days of culture, respectively) and ad-mixed with 1) GFP-MDA-MB-231 human BCCs, or 2) GFP-MDA-231 BCCs double-labeled with CellTracker Orange CMTMR. Forty-eight hours later, these co-cultures were assessed for the presence of gap junction protein expression (Cx43, representative gap junction), and whether they functioned to exchange information using a cellular CMTMR dye transfer assay. Cx43 expression was localized specifically between OBs and BCCs. Interestingly, we observed direct cell-cell dye transfer occurring between BCCs that distributed dye to OBs several cell distances away. Next, we assayed supernatants of OBs and BCCs for the presence of exosomes and exosomal miRs, both known mediators of cell-cell communication. Both OBs and BCCs produced exosomes as characterized by transmission electron microscopy and a Nanosight 500 System. Late differentiated OBs produced over twice the amount of exosomes than did OBs in their growth phase. Given this, we interrogated the contents of the exosomes for miRs that might induce proliferative quiescence in BCCs. miR arrays demonstrated the presence of miR 320a (proliferation), miR302d (reprogramming), and miR 183 (apoptosis, invasive potential) as produced by tumor-conditioned OBs.

Combined, these data suggest that there is extensive crosstalk between OBs and disseminated BCCs in bone. This work highlights the importance of investigating OBs as key players in the generation of a sustainable niche for disseminated BCC survival in bone during BCC dormancy.

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Bone morphogenetic protein-4 inhibits breast cancer metastasis by blocking myeloid derived suppressor cell activity

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Metastatic disease is a major cause of death in women with breast cancer. We report here that bone morphogenetic protein 4 (BMP4) is a potent suppressor of breast cancer metastasis. Mice bearing highly metastatic mammary tumors present with elevated numbers of myeloid derived suppressor cells (MDSC), which are also found in patients and are associated with metastatic disease. The extent of MDSC expansion is markedly reduced upon exogenous BMP4 expression in highly malignant tumors. MDSC are not detected in naïve mice, but can be induced either by treatment with granulocyte-colony stimulating factor (G-CSF) or by secretion of G-CSF from tumors. Both tumor-induced and G-CSF-induced MDSC effectively suppress T cell activation and proliferation, leading to enhancement of metastasis. BMP4 reduces the expression and secretion of G-CSF through inhibition of NFκB activity in human and mouse tumor lines. Since MDSC in breast cancer patients are correlated with poor prognosis, therapies based on activation of BMP4 signaling offer a potential new treatment strategy for patients with progressive breast cancer.
A17 The effects of hypoxia on tumour progression in breast cancer

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Metastasis is a major cause of morbidity and mortality in solid cancers. Thus, delineating the molecular mechanisms that facilitate tumour cell spread is of paramount importance to fully understanding cancer progression and to the development of novel therapeutics. Hypoxia, the state of low oxygen conditions, occurs frequently in solid tumours, such as breast cancer, and is a poor prognostic factor for patient outcome. The expression of Hif-1α, the main mediator of the hypoxic response pathway, and the up-regulation of its target genes have been implicated in tumour growth, invasion, angiogenesis, therapy resistance and metastasis. Despite the importance of the role of hypoxia in tumour progression, there is currently no successful manner in which to prevent, inhibit or reverse its many downstream effects. There are two forms of hypoxia present in a growing tumour: chronic hypoxia and cycling hypoxia. Chronic hypoxia is caused by limitations in oxygen diffusion from abnormal tumour vasculature. On the other hand, the aberrant, repeated and temporary closing and re-opening of blood vessels, leading to fluctuations in oxygen supply, give rise to cycling hypoxia. The effects of these two different forms of hypoxia on tumour cells metastatic potential have not yet been comprehensively studied.

To investigate the contributions from both types of hypoxia, we have developed a model using breast cancer cell lines derived from tumours arising in mice expressing the Polyoma Middle-T oncogene under the control of the MMTV promoter. By exposing cells to 9 days of 20\% O\textsubscript{2} (normoxia), 1\% O\textsubscript{2} (long-term hypoxia) or alternating 24 hour cycles in 20\% O\textsubscript{2} and 1\% O\textsubscript{2} (cyclic hypoxia), we observe that cyclic hypoxia generates tumour cells with greater metastatic potential. These cells have the ability for low-anchorage dependent growth, as shown by their propensity to form a greater number and larger colonies when grown in Matrigel and on low-attachment plates. Cyclic hypoxia-treated cells express increased levels hypoxia response genes, but upon re-exposure to 1\% O\textsubscript{2}, their hypoxic response is dampened. These cells also undergo the Warburg effect, as evidenced by the up-regulation of several glucose metabolism genes. Using RNAseq data, we find that cyclic hypoxia causes the expression of a lung metastasis gene signature. Although these treatments produce no difference in primary tumour growth when orthotopically injected into the mammary fat pad of mice, cyclic hypoxia-treated cells give rise to a greater number of lung metastases. This data suggests that cyclic hypoxia endows tumour cells with greater metastatic potential.
A18 Cooperation of Neurotrophin Receptor TrkB and Her2 in Breast Cancer Cells Facilitates Brain Metastasis

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Metastases are responsible for 90% of all cancer deaths, and patients diagnosed with brain metastasis have a dismal 20% probability of one-year survival. Breast cancer metastasizes to the brain in approximately 40% of patients who have a Her2+ tumor. The “seed and soil” hypothesis framing the current investigation of metastasis is uniquely exemplified by the colonization of the brain by circulating breast cancer cells. Accordingly, therapy for brain metastases should not only employ cytotoxic approaches against the tumor cell, but also perturbation of the microenvironment that facilitates cancer cell growth and resilience.

Despite disseminating to distant organs as malignant scouts, most tumor cells fail to remain viable after their arrival. Previous research shows that the brain’s physiologic microenvironment must become tumor-favorable for successful metastatic colonization by breast cancer cells. The bidirectional interplays of metastatic breast cancer cells and native brain cells are poorly understood and rarely studied.

Astrocytes, prominent glial cells in the central nervous system, are responsible for homeostasis of the brain microenvironment. Furthermore, astrocytes secrete neurotrophins, such as brain-derived neurotrophic factor (BDNF), which are multifunctional growth factors with a crucial role in synaptic plasticity, survival, and cognitive function. BDNF binds to the extracellular domain of the Tropomyosin-Related Kinase B (TrkB) receptor. Therefore, we hypothesize that Her2+ breast cancers express TrkB receptors to exploit BDNF from the brain microenvironment for metastatic colonization. Our results show high expression of TrkB receptor in patient specimens of fresh tissue and cells from neurosurgical resections of Her2+ breast-to-brain metastases (BBMs). Because BDNF expression is highest in the brain microenvironment, paracrine signaling may potentiate tumor cell growth and survival. Indeed, our results show both exogenous and astrocyte-derived BDNF confers a TrkB-dependent cellular proliferation in Her2+ BBMs. In vivo xenograft studies show a delay in brain colonization and metastatic growth with BBM TrkB-knockdowns (BBM-KD).

Because Her2 is an orphan receptor that can heterodimerize with ligand-bound receptors, we looked at the possible interaction between TrkB and Her2 in BBM cells. Our results show colocalization of Her2 and phosphorylated TrkB receptor in BBM tissue and cells. Co-immunoprecipitation and 3-D structural modeling confirm direct binding of Her2 and TrkB receptors. Furthermore, BDNF treatment induces TrkB-Her2 heterodimerization, which is abrogated in BBM-KD. These results suggest that in order to establish a metastatic niche, tumor cells exploit BDNF from the brain microenvironment, resulting in subsequent heterodimerization of TrkB and Her2 receptors and promoting proliferation and colonization.
A19  The Kallikrein-related serine peptidase, KLK4, regulates the TGFβ1 pathway in the tumour-stroma microenvironment in prostate cancer.

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Prostate cancer cells reside in a complex stromal microenvironment often referred to as “reactive” stroma which is a critical component of prostate cancer initiation and progression. The mounting evidence for its critical nature has led to increased interest in this niche as a target for new therapeutic approaches. Cancer associated fibroblasts (CAFs) play a key role in this niche regulating the tumour microenvironment. Factors secreted by prostate cancer cells can ‘activate’ non-malignant associated fibroblasts to become CAFs. KLK4 is over-expressed in both localised and bone metastatic prostate cancer and so has the capacity to act as a paracrine factor on the surrounding stroma. The Aim of this study was to elucidate the role of KLK4 in tumour-stroma cross-talk by identifying its substrates in the prostate cancer lines, LNCaP and PC3 (also derived from a bone metastasis) and in the prostate (stromal) fibroblast line WPMY-1. This was accomplished at the protein level utilising the ‘PROtein TOpography Migration Analysis Platform’ (Dix et al., Cell, 2008). Gene expression changes following KLK4 treatment were also assessed by gene microarray analysis. We identified 50 putative novel KLK4 protein substrates, 11 of which directly interact with the growth factor TGFβ1. Strikingly, the most enriched pathway (based on DAVID analysis, p<0.01) following transcriptome analysis of the KLK4-treated cells was the TGFβ1 pathway. KLK4-treated fibroblasts also expressed elevated levels of a number of genes consistent with a CAF genotype. These findings suggest that KLK4 is a critical regulator of the reactive stromal niche, via the TGFβ1 pathway, and a potential novel therapeutic target for prostate cancer.
A20  Gamma delta T cells and neutrophils conspire together to promote breast cancer metastasis

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Metastatic disease remains the primary cause of death for breast cancer patients. The different steps of the metastatic cascade rely on reciprocal interactions between cancer cells and their microenvironment. Within this local microenvironment, immune cells and their mediators are known to facilitate metastasis formation. However, the precise contribution of tumor-induced systemic inflammation to metastasis and the mechanisms regulating systemic inflammation are poorly understood. Here, we show that tumors maximize their chance to metastasize by evoking a systemic inflammatory cascade in mouse models of spontaneous breast cancer metastasis. Using the K14cre;CdhlF/F;Trp53F/F (KEP) model of invasive lobular carcinoma, we observed a profound expansion and accumulation of neutrophils throughout every organ. We then tested the functional importance of neutrophils in our recently described spontaneous metastasis model that accurately recapitulates each step of the metastatic cascade. This model is based on the transplantation of KEP tumor pieces into the mammary glands of WT recipient mice. These tumor pieces are allowed to grow out, then surgically removed, after which 100% of mice succumb to metastatic disease. Antibody-mediated depletion of neutrophils in this model resulted in a marked decrease in pulmonary metastasis, confirming the pro-metastatic role of these cells.

We then asked the question: how do mammary tumors regulate neutrophil expansion and function? Cytokine analysis of KEP mammary tumors and wild-type mammary glands led us to investigate the IL17 pathway. IL17 was not differentially expressed between tumors and wild-type mammary glands, but IL17 was increased in the serum of mammary tumor-bearing mice. Surprisingly, gamma delta T cells, not CD4+ cells, were the main source of IL17. Neutralizing antibodies against either gamma delta T cells or IL17 resulted in a dramatic reduction of neutrophil expansion and alteration of neutrophil phenotype. In accordance with this observation, K14cre;CdhlF/F;Trp53F/F;Rag1—/— mice lacking an adaptive immune system exhibited reduced IL17 levels, reduced circulating neutrophils, altered neutrophil phenotype and reduced metastasis. Current efforts are underway to determine which tumor-derived factor initiates this systemic inflammatory cascade. Our data indicate that targeting this cancer cell-initiated domino effect within the immune system – the γδT cell-IL17-neutrophil axis – represents a new strategy to inhibit metastatic disease.
A21 Fibroblasts-secreted YKL-40 enhances tumor growth and angiogenesis

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Breast cancer continues to be one of the leading causes of cancer related death in women, and the requirement for better therapies is an unmet clinical need. Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Cancer-Associated Fibroblasts (CAFs) are an activated sub-population of stromal fibroblasts, which have different characteristics in different tumor types and tissue locales. CAFs were shown to facilitate tumor growth by supporting tumor cell growth, enhancing angiogenesis and ECM remodeling. We previously demonstrated that CAFs mediate tumor-promoting inflammation in various mouse and human carcinomas.

YKL-40, also known as CHI3L1, is a secreted heparin-binding glycoprotein, induced specifically during the course of inflammation. In addition, YKL-40 was shown to be expressed and secreted by several types of solid tumors. Although YKL-40 plays a pivotal role in exacerbating the inflammatory processes and in promoting angiogenesis and remodeling of the extracellular matrix, the exact function of YKL-40 in inflammation and cancer is still largely unknown.

Utilizing a transgenic mouse model of de novo mammary carcinogenesis, we found that YKL-40 expression in fibroblasts is up regulated with tumor progression. We show that paracrine signaling from breast tumor cells induces YKL-40 expression in normal mammary fibroblasts (NMF) in vitro. Incubating breast tumor cells with exogenous YKL-40 induces expression of pro-inflammatory genes, up-regulates the MAPK and PI3K pathways, and enhances tumor cell migration in a transwell assay. Moreover, in tumor cell transplantation assays, we show that supplementing breast cancer cells with YKL-40 results in enhanced angiogenesis in mice.

These findings implicate secreted YKL-40 in the crosstalk between tumor cells and their microenvironment, and deepen our understanding of the contribution of mammary CAFs to tumor progression and metastasis.
Defining the impact of TGFβ inhibition on the metastatic prostate cancer-bone microenvironment using an integrated biological/mathematical modeling approach.

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Prostate metastases progress in the skeleton by manipulating bone forming osteoblasts and bone resorbing osteoclasts. Transforming growth factor beta (TGFβ) is a key factor driving prostate cancer-bone interaction and we observed, using semi-quantitative immunohistochemical analysis, that TGFβ signaling is significantly higher in prostate cancer cells relative to the surrounding bone stroma in human specimens. These data and others suggest that targeted inhibition of TGFβ would be an efficacious approach for the treatment of prostate to bone metastases. However, TGFβ can have differential effects on the bone microenvironment making the impact of TGFβ inhibition on a multicellular level difficult to determine, especially with traditional biological approaches. To address this dilemma, we have utilized the power of computational modeling and taken an integrated approach where biological results inform a predictive mathematical model, allowing for exploration of complex multicellular interactions mediated by TGFβ. The model was parameterized with empirical and published data. Simulations of the prostate cancer-bone microenvironment over a 208 day period generated a clinically relevant pathophysiological model with the following outputs; 1) that TGFβ is key for coordinating prostate cancer-induced phases of osteolysis and osteogenesis and; 2) that mesenchymal stem cells (MSCs) are crucial for the osteogenic component of the disease. To test these in silico generated hypotheses we performed biological studies using a TGFβ antibody-based inhibitor (1D11). Our results demonstrated: 1) TGFβ in prostate cancer cell conditioned media significantly contributes to MSC and osteoblast precursor recruitment, 2) using an intratibial model of prostate cancer-induced osteogenesis (PAIII), we identified that TGFβ inhibition (1D11; n=10) significantly impacted PAIII growth compared to controls (13C4; n=9; p<0.05) and; 3) TGFβ inhibition reduced both prostate cancer induced osteolysis and osteogenesis via μCT and histomorphometry. Histological analyses of in vivo PAIII specimens were also comparable to the outputs of the mathematical model illustrating the usefulness of integrated approaches in defining the impact of potential therapies on the metastatic bone microenvironment. In conclusion, these data suggest TGFβ inhibition as a viable approach for the treatment of prostate to bone metastases.
A23  What metastatic breast cancer patients want, need, know and don’t know

CJ (Dian) Cornelussen-James

METAvivor Awareness, Research and Support

Background: This two-part study attempted to build a worldwide profile of metastatic breast cancer (MBC) patients and identify wants and needs. Method: A 2011 lifestyles and support survey and a 2014 MBC patient-oriented research survey (2014) were distributed to MBC patients through cancer organizations, patient networks and Facebook. Results: A combined total of 1,087 respondents from 22 countries including Canada (Can), Asia Pacific (AP-largely Australia and New Zealand), European Union (EU) and the United States (US) represented a relatively young cohort (32% aged 15-45; 42% aged 46-55). (The Russian (1), Israeli (1) and South American (1) cohorts were too small for regional statistics.) Disease status was relatively good (NED or Remission: Can 13%; AP 14%; EU 17%; US 20%. Stable: Can 44%; AP 53%; EU 56%; US 52%). Most were within five years of diagnosis (Can 84%; AP 50%; EU 83%; US 62%). A significant number (40%) felt "outcast" and "isolated". Regions varied greatly as to whether hospitals offered MBC patient support (Yes: Can 67%; AP 33%; EU 0%; US 39%). The majority of respondents sought and found preferable support elsewhere (Can 60%; AP 62%; EU 58%; US 72%). Online support was popular (Can 82%; AP 85; EU 86%; US 66%) in part because it was available 24/7. The paucity of public knowledge of metastasis took a heavy toll. “Metastasis is a scary thing, one that needs to be presented to society as a whole, not just stumbled upon after your cancer has metastasized.” Optional comments from all regions revealed a great need for face-to-face support, expert counseling on medical and emotional issues and increased research. US respondents further expressed a great need for disability, insurance and financial advisors. When asked to select one top priority, 75% chose scientific research. The vast majority (80%) reported having a reasonable understanding of research; 96% endeavored to remain up-to-date. A minority (24%) had scientific backgrounds; others had knowledgeable acquaintances or followed cancer center/organization reporting. The majority (74%) were unaware, some expressing outrage that the term “metastasis research” included “prevention of” and “progression to” metastasis. Comments: “Reclaim metastasis”; “if it’s prevention call it prevention, mets is mets”. Many (69%) were unaware there were societies for metastasis researchers; 86% had never heard of the Metastasis Research Society (MRS). Confusing messaging didn’t help. The term “the cure” was seen as meaning “elimination of all breast cancer at any stage” (35%); “prevention of all new cases of breast cancer” (21%); “prevention of MBC” (7%); “longevity, quality of life and no death for those with MBC”(7%) and “organizations use the term in differing ways” (30%). Of the 247 taking the 2014 survey, almost half took advantage of an offer to send a message to MRS-Heidelberg (handouts in Heidelberg). Conclusion: It is widely understood that patients want long, healthy lives. This will only happen with increased research for which a greater share of the funding pie is paramount. Large scale, patient-driven public demand could help, but patients must first gain a thorough knowledge of all the players and at present, the MRS is largely unknown to the MBC community. Rectifying this is critical for stage IV, patient-oriented research to move forward.
A24 Germline variation modulates susceptibility to metastasis in a mouse model of prostate tumorigenesis


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Prostate cancer typically runs an indolent course with most men, which is reflected in the low prostate cancer mortality; in 2013, over 233,000 new cases were diagnosed in the US with approximately 29,000 men succumbing to unrelated diseases. The vast majority of such prostate cancer-related deaths are a direct consequence of metastasis. We hypothesize that germline variation influences susceptibility to metastasis and other indicators of disease aggressiveness in prostate cancer. Identification of polymorphic germline metastasis susceptibility genes will allow physicians to accurately identify the subset of men at risk for the fatal disease forms. The goal of this work is to identify such genes using the C57BL/6-Tg(TRANMP)8247Ng/J (TRAMP) mouse model of neuroendocrine prostate cancer.

Genetic loci modulating susceptibility to aggressive prostate cancer were identified using a quantitative trait locus (QTL) mapping approach in an F2 intercross population involving TRAMP and NOD/ShiLtJ mice, with the latter strain being highly susceptible to aggressive disease development. Transgene-positive F2 male mice (n=232) were genotyped using a linkage panel of 1,449 germline SNPs and modifier loci were characterized using j/qtl. This led to the identification of 5 QTLs associated with metastasis susceptibility and 5 QTLs with growth and invasion of the primary tumor. QTL candidate genes were identified by performing microarray analysis of all TRAMP x NOD/ShiLtJ F2 primary tumors (n=122). Aggressive disease susceptibility genes were nominated through a combination of gene expression-disease trait correlation and expression QTL (eQTL) mapping. High priority candidate genes were those that demonstrated both high levels of expression-trait correlation and a cis-eQTL. Through this process, 27 candidate aggressive disease susceptibility genes were identified.

The role of these candidate genes in aggressive forms of human prostate cancer was investigated using cBioPortal. Given that the expression levels of these genes are a key determinant of disease aggressiveness in the TRAMP mouse, we hypothesized that the expression of the human orthologs of these genes would be dysregulated in tumors associated with a poorer prognosis. Accordingly, dysregulated expression of 9 candidate genes in primary prostate tumors was consistently associated with a reduced distant metastasis-free survival in two human prostate cancer gene expression datasets.

The further relevance of these genes to aggressive human prostate cancer is currently being investigated through analysis of SNP frequencies in publically-available human prostate cancer genome-wide association study cohorts and through functional analysis of individual candidate genes. This approach will facilitate the identification of novel germline factors driving metastasis susceptibility and allow for new insights into this deadly form of prostate cancer.
A25  Therapeutic targeting of the transcription factor MYB in breast cancer metastasis with a DNA vaccine

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Breast cancer is a heterogeneous disease with 3 main histo-pathologies based upon hormone receptor status. From an immunological perspective this heterogeneity offers a large antigenic pool, the majority are over expressed self-peptides and as such are weakly immunogenic, requiring tolerance to be overcome to illicit immune responses.

MYB is a transcription factor important in neuronal, hematopoietic, gastrointestinal and breast development. It can promote proliferation, suppress or induce differentiation and has recently suggested a role in regulating immune suppression. Both our lab and others have shown that MYB is also important in all 3 histo-pathologies of breast cancer. Data from spontaneous, BrCa1 and BrCa2 tissue micro arrays suggests that it is expressed in the majority (>95%) of breast cancers. The use of transgenic mouse models has shown it is critical for the transformation of MMTV-PyMT and MMTV-Neu mouse breast cancers. Furthermore an up regulation of MYB can be observed in metastatic lesions compared to primary disease in preclinical breast cancer models.

The increased expression of MYB in metastasis makes it an ideal target for therapy, however the intra-nuclear location of MYB makes targeting it difficult. Thus far immunotherapy has been largely focused on targeting tumor surface antigens, such as Her2. MHC class I presentation is classically linked to immune responses to intracellular pathogens, whereby intracellular pathogen antigens are presented on the cell surface. Thus by using MHC class I presentation it becomes possible to target over expressed intracellular self antigens such as MYB.

With this knowledge we have developed a DNA vaccine that encodes the full length MYB cDNA, to overcome class I and class II MHC restrictions. The coding of two adjuvant peptides derived from tetanus toxoid (P2 and P30) at each end of the cDNA allow for both the enhancement of immune responses and allows tolerance to be broken. Previously we have shown that prophylactic vaccination elicits a CD8-dependent attenuation of disease in the 4T1.2 breast cancer metastasis mouse model. Here we progress to show data that this attenuation can also be achieved therapeutically in the E0771-LMB breast cancer metastasis mouse model following primary tumor resection.
A26  Tissue inhibitor of metalloproteinases-1 induces a pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes

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Tissue inhibitor of metalloproteinases-1 (TIMP-1) recently emerged as a pro-metastatic factor highly associated with poor prognosis in a number of cancers. This correlation seemed paradox as TIMP-1 is best described as inhibitor of pro-tumourigenic matrix metalloproteinases. Only recently, TIMP 1 has been revealed as a signalling molecule which can regulate cancer progression independent of its inhibitory properties. In the present study, we demonstrate that an increase of both exogenous and endogenous TIMP-1 led to upregulation of miR-210 in a CD63/P110/P85 PI3K-signalling and AKT phosphorylation. It also led to increase of HIF-1α protein levels positively correlating with HIF-1-regulated mRNA expression and upregulation of the microRNA miR-210. Downstream targets of miR-210, namely FGFRL1, E2F3, VMP-1, RAD52, and SDHD were decreased in the presence of TIMP-1. Upon the overexpression of TIMP-1 in tumour cells, miR-210 was accumulated in exosomes in vitro and in vivo. These exosomes promoted tube formation activity in HUVEC cells which was reflected in increased angiogenesis in A549L-derived tumour xenografts. Activation and elevation of PI3K, AKT, HIF-1A, and miR-210 in tumours additionally confirmed our in vitro data. This new pro-tumourigenic signalling function of TIMP-1 may explain why elevated TIMP-1 levels in lung cancer patients are highly correlated with poor prognosis.
A27 Identification of common disrupted protein networks in metastasis across multiple cancer types

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To generate metastases, carcinoma cells progress through a series of changes, many of which involve processes mediated by molecular interactions between proteins. Large scale protein-protein interaction networks are now available covering upwards of two thirds of human proteins, and analysis of such networks can identify the disruption of specific mechanisms regulating behavior within or between cells. In order to shed light on the fundamental processes driving metastasis, we have applied an integrated network and transcriptomic analysis across multiple types of cancer with clinical annotation of local and metastatic disease. Taking microarray data from breast, prostate and pancreatic cancer, we identify genes differentially-expressed between patients with local or metastatic disease in each experiment. Independently, we use a knowledge-base of protein-protein interactions to build a network for proteins annotated with processes implicated in the metastatic cascade. We integrate differentially-expressed genes with this network and identify sub-networks significantly disrupted in each cancer, as well as those disrupted in all three. The network intersection suggests common pathways of metastasis in tumours. The results implicate a starvation response in resource depleted tumour masses, highlighted by processes including autophagy hypoxic response and known triggers of epithelial-mesenchymal transition. These results have implications for use of tumour-starving drugs in treatment. Further experiments to determine the reproducibility of our results and the significance of starvation in triggering metastasis are required, including both a deeper analysis of pancreas, prostate, and breast cancers, and wider analysis of other carcinomas.
A28  The role of MEK signaling in driving mesenchymal transition in cell line models of human breast cancer metastasis

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Epithelial to mesenchymal transition (EMT) is the process whereby sessile, polarised epithelial cells alter the expression of key adhesion and regulatory molecules, and gain the ability to survive and migrate as single cells. While this process occurs normally in development we now recognize that metastasis has many elements in common with developmental EMT, which is subverted by carcinoma cells to allow metastatic spread. Cancer cells rarely undergo a full conversion to the mesenchymal phenotype, and instead appear to occupy a range of positions along a phenotypic spectrum between epithelial and mesenchymal states. The Mitogen Activated Protein Kinase (MAPK) signaling cascade, which signals through MEK and ERK proteins, has previously been implicated in the regulation of EMT. In this work, we have used previously established breast cancer derived cell line models of epithelial-mesenchymal plasticity and applied RNA sequencing to measure transcriptional changes that occur as cells transition to a more mesenchymal phenotype. We have identified co-ordinated changes in the expression of genes in many, although not all, MEK-related signaling pathways, and delineated modulation in the abundance of key miRs implicated in breast cell differentiation and EMT. Collectively, our results demonstrate that different MEK related signaling pathways are altered by the induction of EMT in our cell line models, depending on the cell line’s initial position on the epithelial-mesenchymal spectrum, and the type of stimulus used to trigger transition.

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Co-evolution of tumor microenvironment revealed by QDs-based multiplexed imaging

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One urgent task is to develop novel approaches to let us “see” the rich hidden information in cancer microenvironment simultaneously so as to further elucidate the complex mechanism of cancer invasion and metastasis. Quantum dots (QDs)-labeled molecular probes are promising platforms to simultaneously study several subtle changes of key biomolecules, because of their unique optical and chemical properties. With this newly developed molecular imaging technology and multispectral analysis software, our group systematically studied the role of major components in tumor microenvironment, including type IV collagens, macrophages and tumor neo-vessels, and developed a comprehensive model of tumor invasion based on the novel results. This new model of cancer invasion could better account for the difficult clinical problem of cancer invasion, metastasis and recurrence, and suggest new strategies to curb cancer progression. This so-called “pulse mode” of cancer invasion has been well received (Biomaterials. 2013;34(34):8708-17), and a new field of tumor microenvironment research has been developed (Tumor biology. PMID: 24338768).

In addition, in the study of gastric cancer (GC), we find that there are great differences of tumor macrophages infiltration and angiogenesis between poorly- and well-differentiated GC. Although tumor stroma is less in poorly-differentiated than well-differentiated GC, the ‘tumor invasion unit’ consisted of tumor cells, tumor associated macrophages and tumor neo-vessels are much more, and are distributed uniformly across an entire tumor (Biomaterials. 2014;35(13):4125-32). Typically, they are regarded as single structures scattered throughout the carcinoma, so the ‘invasion field’ consisted of ‘tumor invasion units’ is much stronger.

In summary, the established approach for QDs-based in situ multiplexed imaging of clinical cancer tissues permits the visualization of the temporal-spatial process of the co-evolution of cancer cells and their microenvironment. Such an approach could help us observe cancer invasion from the perspectives of not only cancer cells but also their microenvironment, gaining new insights into this complex and critical cancer event.
A30 Mechanistic dissection of the CCR4-NOT complex deadenylase, Cnot7, as a catalytic-activity dependent driver of tumor metastasis

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Emerging evidence has revealed that transcriptome-level alterations modulate the metastatic capability of tumor cells. Yet despite great interest in the contribution of transcriptional regulation to cancer metastasis, relatively little attention has been paid to the role of RNA stability, which also contributes to transcriptome-wide RNA abundance. Deadenylation is thought to be the rate limiting step of mRNA degradation and is thus a primary determinant of RNA stability. We previously interrogated global transcription networks to identify the CCR4-NOT complex, a major deadenylase in metazoan cells, as a modifier of metastasis. As an enzymatic process, deadenylation represents an unexplored actionable target for the treatment or prevention of metastatic disease.

We show here that knockdown of the CCR4-NOT deadenylase Cnot7 dramatically suppresses tumor cell metastatic capacity, without impacting tumor growth, in an orthotopic transplant model of metastasis. Consistent effects were also observed in the MMTV-PyMT autochthonous model for metastatic progression, in which monoallelic knockout of Cnot7, corresponding to a 50% reduction of Cnot7 expression, suppressed both metastasis count and incidence without impacting tumor mass.

While overexpressing wild type Cnot7 enhanced metastasis in vivo, its deadenylase inactive point mutant had no effect, suggesting that Cnot7 deadenylase activity is required to promote metastasis. We further identified an isoform of Cnot7 (Cnot7Δ6) that lacks an exon containing 2 residues essential for deadenylase activity. Cnot7Δ6 suppressed metastasis in vivo, consistent with dominant negative activity, supporting the deadenylase dependency of Cnot7-mediated metastasis promotion.

Transcriptome profiling coupled with computational and epidemiologic analysis revealed that Cnot7-regulated transcripts constitute a metastasis suppressive expression program enriched for Cpeb3 and Nanos2 binding motifs (p<10^{-66} and p<10^{-101}, respectively). Cpeb3 and Nanos2 are sequence specific RNA binding proteins that recruit Cnot7 to transcripts via Tob1 and Cnot1 scaffolds, respectively. Intriguingly, Cpeb3, Cnot1, and Tob1 predict distant metastasis free survival in human breast cancer cohorts.

We generated tumor cells expressing Cnot7 mutants that specifically disrupt Cnot7 binding either to Tob1 or Cnot1. Expression of either mutant showed a phenotype more metastatic than control but less metastatic than overexpressing wild type Cnot7. This suggests that both Tob1 and Cnot1 contribute to pathways endowing transcript specificity in Cnot7-mediated metastasis promotion.

These observations support a model for Cpeb3-Tob1-Cnot7 and Nanos2-Cnot1-Cnot7 ternary complexes that bind metastasis suppressive transcripts. We hypothesize that once bound, Cnot7 initiates the degradation of these transcripts, tipping the balance of the tumor cell transcriptome to drive metastasis. Our results identify the CCR4-NOT deadenylase Cnot7 as a pleiotropic driver of metastasis via post-transcriptional control of RNA abundance and establish CCR4-NOT deadenylases as a novel class of actionable targets in the treatment and prevention of metastatic disease.
A31  FOXF2 Suppresses Epithelial-mesenchymal Transition via Negatively Regulates Transcription of FOXC2 in Basal-like Breast Cancer

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Forkhead box F2 (FOXF2) and FOXC2 belong to Forkhead box transcription factor family. FOXC2 is recognized as an epithelial-mesenchymal transition (EMT)-inducer and FOXC2 overexpression promotes metastasis of basal-like breast cancer (BLBC). Our previous study found that FOXF2 function as an EMT-suppressor and FOXF2 deficiency promote metastasis of BLBC. The relationship between the opposite EMT-related transcription factors of FOXF2 and FOXC2 has not been reported. In this study we defined that FOXC2 is a direct transcriptional target of FOXF2 and FOXF2 negatively regulates FOXC2 transcription. Functionally, we showed that FOXC2 is essential for FOXF2-regulated EMT phenotype, migration and invasion behavior, as well as chemical drug resistance of BLBC cells. Additionally, we revealed a significant negative correlation between the FOXF2 and FOXC2 mRNA levels in triple-negative breast cancer (TNBC) tumors. The TNBC patients in the FOXF2\textsubscript{high}/FOXC2\textsubscript{low} and FOXF2\textsubscript{low}/FOXC2\textsubscript{high} groups had the best and poorest DFS, respectively. The patients in the FOXF2\textsubscript{high}/FOXC2\textsubscript{high} and FOXF2\textsubscript{low}/FOXC2\textsubscript{low} groups had moderate DFS. In summary, we found that FOXC2 is a transcriptional targeted of FOXF2. FOXF2 suppresses EMT through negatively regulates transcription of FOXC2 in BLBC. The combined FOXF2 and TWIST1 mRNA expression levels might serve as an effective prognostic indictor and guide tailored therapy for TNBC patients.
A32  Molecular characterization and biological role of circulating tumor cells: a window into metastasis biology from breast cancer xenograft models.

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How breast cancer (BC) cells disseminate from primary tumor (PT) into blood circulation or lymphatic system and give rise to distant metastases is still under investigation and represents a field of interest both to cancer biologists and clinical oncologists. Circulating tumor cells (CTC) characterization offers an opportunity to better understand this process and might help in generating biomarkers for identification of patients at high risk of relapse or progression. However, few CTC experimental models are currently available.

We investigated the gene expression profile (GEP) of CTCs isolated by filtration (ScreenCell® MolecularBiology kit) from the blood of NOD/SCID mice xenotransplanted with MDA-MB-231 cells (MDA231) into the mammary fat pad (mfp). Animals were sacrificed after 80 days from cells injection and GEPs were generated using the WG-DASL HT12 v4 assay (Illumina). GEP from CTC biological replicates was compared with those obtained from frozen sections of primary tumor (PT) nodules and of metastatic foci at lung (LUNG) and lymph-nodes (LNs).

Unsupervised clustering of the most variable genes (209 with IQR > 99th percentile) revealed that CTCs present a quite peculiar GEP as they clustered separated from the other tumor lesions and from the parental cell line. Indeed, a CTC-specific gene signature including 65 up-regulated (log₂Fold Change [FC]≥1.5) and 122 down-regulated (log₂FC≤-1.5) genes in CTC compared to the other tissue compartments (P<adj<0.0001) was identified. Interestingly, Gene Ontology (GO) analysis revealed that the cluster of up-regulated genes was enriched in 5/7 (71%) GO terms referring to development, whereas the cluster of down-regulated genes was enriched in 23/32 (72%) GO terms referring to chromatin organization/assembly. Further sub lists were generated in order to identify candidate genes putatively involved in the earliest phases of metastasization, thus particular attention was paid to those differentially expressed between CTC and PT. On the whole, 81 genes were significantly up-regulated and 106 genes were significantly down-regulated in CTC compared to PT.

These data 1) demonstrate for the first time that CTCs obtained from a BC xenograft model have a different transcriptional profile compared to their parental cell line and tumor cells grown in mfp, disseminated to LN or colonizing lungs and 2) provide experimental evidences of the transcriptional reprogramming required during tumor cell dissemination. Analyses are ongoing to better define the CTCs molecular profile along with functional validation experiments in order to dissect mechanisms that orchestrate invasion, dissemination, dormancy and metastatic outgrowth.
A33 The roles of ASC in cancer cell metastasis

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Dysregulation of both actin cytoskeleton and kinases have been implicated in cancer metastasis. Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) has been reported as one of the pro-apoptotic molecules that is epigenetically silenced in several human metastatic cancers. ASC is well recognized as an adaptor protein to form various inflammasomes, and has a crucial for caspase-1 activation and secretion of interleukin (IL)-1β and IL-18 in innate immune cells. However, the role of ASC in the regulation of the tumor progression remains elusive. Here, we investigated the roles of ASC in cancer progression, i.e. acquisition of metastatic ability of murine melanoma cell line. To determine the importance of ASC for metastasis and cell motility in vivo and in vitro model system, we stably knocked down ASC in melanoma cell lines using retroviral transduction of shRNA. Loss of ASC increased the motility of B16 melanoma cells in a wound-scratch assay, and caused higher metastatic ability to B16 cell line in the experimental lung metastasis model. These results suggested that ASC negatively regulated tumor cell motility and metastasis by the modulation of the actin cytoskeleton. Furthermore, little information is available for the signaling pathway regulated by ASC in tumor cells. To define the relevance of ASC in metastasis, we examined several signaling pathways known to be involved in cancer metastasis. In comparison with control cells, the phosphorylation levels of Erk and Src were higher in ASC knocked down cells, but it was not detected that the phosphorylation of JNK, p38, and NFkB p65 in both cell types. Interestingly, we also observed that invadopodia formation was markedly enhanced in ASC knockdown cells. It is reported that Erk and Src-mediated signaling activity is elevated in numerous metastatic cancers, and these kinases leads to promote invadopodia formation and cell invasion. Collectively, our findings demonstrated that ASC contributed to the cancer progression and metastasis through a modulation of several signaling pathways.
A34  Silencing of membrane-bound serine protease inhibitor, HAI-1, enhances metastatic capability of pancreatic cancer cells in mouse models

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Hepatocyte growth factor activator inhibitor type 1 (HAI-1), encoded by the *serine protease inhibitor Kunitz type 1 (SPINT1)* gene, is a transmembrane protease inhibitor that is believed to regulate the activities of various membrane-associated serine proteases on surface of epithelial and carcinoma cells. Matriptase is one of the most important targets of HAI-1, and is involved in activation of protease activated receptor-2 (PAR-2). We reported that S2-CP8, a highly metastatic subline of human pancreatic ductal adenocarcinoma cell line, SUIT-2, showed significantly decreased expression of HAI-1. To analyze the roles for HAI-1 in metastatic capability, we performed stable knockdown of HAI-1 in SUIT-2 and forced expression of HAI-1 under the control of a tetracyclin-regulated promoter in S2-CP8 cells. In vitro invasion assays, an inhibitory effect of HAI-1 is revealed. Furthermore, matriptase activity was suppressed by the expression of HAI-1, and the enhanced invasiveness in the absence of HAI-1 was decreased by knockdown of matriptase by 81% and of PAR-2 completely. PAR-2 antagonist also suppressed the invasion, thus, matriptase-mediated PAR-2 activation is considered to be involved in HAI-1 loss-induced invasion of S2-CP8 cells. Next, we examined the roles of HAI-1 in nude mouse models in vivo. In an experimental pulmonary metastasis model with tail vein injection, metastatic colonization in lungs was apparently increased in the HAI-1 knockdown SUIT-2 cells, and pretreatment with recombinant HAI-1 reduced the metastasis. In a spontaneous metastasis model with pancreatic implantation, mice treated with doxycycline to induce HAI-1 expression did not develop metastasis although approximately 50% of the control mice developed liver and/or lung metastasis. These data suggest a pivotal role of the regulation of cell surface serine proteases by HAI-1 in metastatic spreading of pancreatic ductal adenocarcinoma cells.
A35  Response of Melanoma-Bearing Normal and Alcoholic Mice to Sunitinib Malate and ALT-803 Treatment


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The incidence of melanoma continues to escalate, and alcoholism increases melanoma morbidity and mortality. Studies in mice indicate that chronic alcohol intake increases IFN-γ-producing CD8T cells exhibiting a memory phenotype (CD44+) in the early stage of subcutaneous (s.c.) B16BL6 melanoma development; however, this response rapidly decays. Alcohol decreases IL-15-producing cells. IL-15 is a proliferation and survival factor for CD8+ T cells. The interaction between alcohol and melanoma can increase myeloid derived suppressor cells (MDSC), which inhibit CD8+ T cell proliferation and activation. Herein, we examined the single and combined effects of a novel IL-15/IL-15 receptor alpha complex, ALT-803, to sustain CD8+ T cell proliferation and activation and Sunitinib, which inhibits MDSC, on primary melanoma growth and metastasis. Both drugs are being evaluated separately in human melanoma clinical trials. Female C57BL6 mice fed a solid diet and given continuous water or 20% w/v alcohol were inoculated with B16BL6 melanoma s.c. after 3 months. Treatment consisted of daily injections of 40 mg/kg Sunitinib and/or weekly injections of 0.2 mg/kg ALT-803 once tumors were palpable. Tumor growth, survival and metastasis were determined. CD11bGr-1int MDSC, CD8+ T, CD44+CD8+ T, and IFN-γ-producing CD44+CD8+ T cells were analyzed over time in a similarly treated cohort. Sunitinib alone and in combination with ALT-803 decreased tumor growth compared to untreated controls. The effect was greater in the alcohol + Sunitinib group. Sunitinib increased survival and in combination with ALT-803 survival was extended. Alcohol inhibited lymph node and lung metastasis. Sunitinib increased metastasis in all groups. ALT-803, which had no effect on primary tumor growth, inhibited metastasis in both water and alcohol drinking mice. The number and percentage of MDSC increased over time in the spleen and peripheral blood of untreated water mice and in the peripheral blood of untreated alcohol mice. The percentage, but not the number of MDSC increased over time in the spleen of untreated alcohol mice. Sunitinib alone and in combination with ALT-803 had a modest effect in reducing the numbers of MDSC in the peripheral blood and spleen of water mice, and was relatively ineffective in alcohol mice. Initially, ALT-803 alone and in combination with Sunitinib greatly increased CD8+ T cells and CD44+CD8+ T cells in the spleen with a similar pattern in peripheral blood. The treatments also increased IFN-γ-producing CD44+CD8+ T cells, examined only in the spleen. Repeated injections of ALT-803 alone and in combination with Sunitinib were less effective as evidenced by lower levels of these cell phenotypes. The treatments sustained the CD8+ T cell phenotypes in water mice but not in the alcohol mice. Further studies to investigate additional parameters are warranted to define the mechanisms underlying the treatment results. Supported by NIH Grants R21AA022098 and K05AA017149 to GGM and HZ and NSF pre-doctoral fellowship DGE-1347973 to KAG.
A36  The ets transcription factor Elf5 drives metastasis via angiogenesis and recruitment of Gr-1+CD11b+ myeloid derived suppressor cells in luminal breast cancer.

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Key pathways driving normal mammary development are often hijacked and subverted by the carcinogenic process. This is the case for the ets transcription factor Elf5, a master regulator of mammary alveolar development that specifies the formation of the estrogen receptor negative (ER-) secretory lineage during pregnancy, the epithelium responsible for milk production during lactation. We have recently discovered that Elf5 acts during the specification of the basal breast cancer subtype by opposing estrogen action via direct transcriptional repression of FOXA1. This mechanism is also apparent in luminal breast cancer cells that have become resistant to anti-estrogen therapy. Basal and estrogen resistant breast cancers are characterised by a higher risk of metastasis and poor prognosis. Using our Elf5-inducible MMTV-PyMT mouse mammary tumour model and the human breast cancer MDA-MB-231 cells, we demonstrate that Elf5 regulates epithelial-to-mesenchymal transition (EMT), driving an epithelial status resulting in impaired cell invasion and distant seeding. At the same time, however, Elf5 orchestrates profound changes in the tumour microenvironment that largely override these cell autonomous effects, leading to a dramatic increase of pulmonary metastases. Elf5 over-expressing tumours exhibit increased angiogenesis and hemorrhage indicating abnormal vascular reorganization. Interestingly, gene profiling shows that lactation genesets are up-regulated in Elf5 overexpressing tumour cells indicating a reminiscence function of Elf5 still acting in cancer cells, as lactation cannot proceed, involution immediately precedes with the up-regulation of involution related inflammation genesets, including presence of monocytes and interferon response. Neutrophil recruitment characteristic of early stages of involution is co-opted in the tumours, thus Elf5-driven chronic inflammation is associated to an expansion of pro-tumorigenic neutrophils. These neutrophils, also known as tumour infiltrating Myeloid Derived Suppressor Cells (MDSC) (CD11b+/Gr1+), consequently suppress CD8+ T-Cells inducing immune tumour tolerance, a major mechanism of tumour microenvironment-induced metastasis in PyMT tumours. In luminal A breast cancer patient cohorts, cytoplasmic staining of Elf5 correlates with poor prognosis and we have identified lactation and inflammation signatures associated with Elf5 expression in this subtype using The Cancer Genome Atlas (TCGA) database.

Our discovery indicates that an anti-ELF5 therapy may act to maintain sensitivity to antiestrogens and simultaneously suppress the metastatic phenotype in luminal A breast cancers. Patterns of Elf5 expression may provide a marker predicting antiestrogen-insensitive metastasis in luminal breast cancer.
A37  SSeCKS/AKAP12 controls metastasis through multiple organ- and route-specific tumor/microenvironment interactions.

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There is growing appreciation for the role of the senescence-associated secretory phenotype (SASP) in promoting metastasis progression through the secretion of inflammatory factors that activate a local microenvironment, thereby allowing tumors to hijack SASP pathways normally involved in wound healing and aging. However, little is known about the pathways controlling metastasis-promoting SASP. Recent studies suggest that metastasis is promoted by the formation of premetastatic niches (MN), which upregulate injury-related markers in response to tumor-secreted factors. Mice genetically deficient for the SSeCKS/AKAP12 metastasis suppressor (“KO”) exhibit 3- to 8-fold higher levels of peritoneal or lung metastasis, respectively, compared to WT hosts following i.v. injection of B16F10 melanoma cells. In contrast, s.c. tumor growth rates did not differ in KO vs. WT hosts. Enhanced lung metastasis correlated with increased tumor cell adhesion to lung endothelial cells (LEC) in a P/E-selectin-dependent manner. B16F10 showed increased adherence to KO- vs. WT-LEC in vitro, correlating with and depending on increased P/E-selectin expression. The siRNA-mediated knockdown of AKAP12 in HUVEC endothelial cells was sufficient to induce increased E-selectin expression and B16F10 adhesion. Although there was no difference in the in vitro adhesion of B16F10 to WT- or KO-lung fibroblasts (LF), pre-incubation of WT-LEC with conditioned media (CM) from KO-LF induced more E-selectin expression and B16F10 adhesion than with CM from WT-LF. The enhanced CM effect correlated with the upregulation of Vegf, Heregulin and Epimorphin/Syntaxin-2 in KO-LF. Co-injection of WT hosts with B16F10 plus KO- vs. WT-LF resulted in increased i) early co-localization of LF with tumor cells in the lung, ii) LF association with leaky vasculature (based on FITC-dextran labeling), and iii) lung metastasis formation. KO fibroblasts exhibit increased Rb-dependent premature senescence and SASP factor secretion caused by hyperactive PKCa and ε, and Src/STAT3 signaling due to the loss of SSeCKS scaffolding activity for PKC and Src. This correlated with increased levels of MEK/ERK activity, and increased expression p16Ink4a, p21Cip1 and senescence-associated β-galactosidase in KO- vs. WT-LF. Taken together, our data strongly suggest that SSeCKS/AKAP12 normally suppresses formation of the MN by attenuating PKC and Src/STAT3 pathways that regulate endothelial cell adhesion proteins and SASP-like crosstalk factors expressed by local stromal cells. The data support the use of PKC and/or Src/STAT3 antagonists to therapeutically prevent or treat metastasis by targeting MN formation.
A38  The Role of Hypoxia and Inflammation in the Tumor Microenvironment of Colon Carcinoma


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Colon carcinoma represents one of the most frequent cancers, associated with high mortality, mainly due to liver metastasis. Development of hypoxic zones within the tumor is linked to poor prognosis.

We established an orthotopic syngeneic model of colon carcinoma by injecting MC-38 cells constitutively expressing GFP into the cecum of C57BL/6 mice. We injected MC-38GFP cells with silenced HIF-1α expression. Mice developed primary tumors after four weeks of the injection. Tumors were harvested at this time point for further analysis using flow cytometry and histology.

The preliminary results show that the percentage of neutrophils was decreased in primary tumors of mice injected with silenced HIF-1α MC-38GFP cells. The percentage of macrophages in HIF-1α tumors was increased. In addition, differences in phenotype (M1 markers versus M2 markers) have been detected which is being confirmed by cytokine profiling. Histological analysis of pimonidazole staining reveals that Mock tumors have an increased percentage of hypoxic zones compared to HIF1α-KD tumors. Moreover, we are investigating the development of hypoxic regions in tumors by using live imaging approaches. To investigate the role of leukocytes in tumor development, we are studying the effect of induced colon inflammation in tumor growth.

Based on deep sequencing data of MC-38GFP cells and protein-protein-interaction network analysis, we are investigating the link between inflammation and hypoxia in tumor development and metastasis and validating identified targets.
A39 Targeted expression profiling of single disseminated cancer cells isolated from bone marrow of prostate cancer patients

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Detection of disseminated cancer cells (DCC) in the bone marrow (BM) of prostate cancer (CaP) patients without distant metastasis (M0) is associated with poor prognosis. DCC comprise founder cells of later arising lethal metastasis, and they are targets for adjuvant therapies, aiming to delay or prevent outgrowth of macrometastasis. Therefore, detailed molecular characterization of DCC is urgently needed. Detection of DCC using staining against surface protein, epithelial cell adhesion molecule (EpCAM), enables isolation of individual viable EpCAM+ cells, from which it is possible to isolate mRNA and genomic DNA (gDNA) that can subsequently be subjected to comprehensive molecular analysis. However, such analysis is hampered by the low frequency of EpCAM+ DCC (on average 1 DCC per 10^6 BM cells), and the existence of EpCAM+ hematopoietic cells, suggested to belong to erythroid lineage. In this study, we applied combined analysis of genome and transcriptome of single cells, aiming to distinguish between EpCAM+ DCC and EpCAM+ hematopoietic cells. We screened BM samples of 105 M0 CaP patients, 2 M1 CaP patients and 18 healthy donors (HD). After screening on average 2 x 10^6 cells per BM sample, EpCAM+ cells were detected in 62% of M0 CaP samples, and 56% of HD samples (p=0.6), as well as in both M1 patients. Although EpCAM+ cells from CaP patients’ BM tended to be stained brighter compared to EpCAM+ cells from HD, DCC and hematopoietic cells cannot be distinguished by the intensity of EpCAM staining, as supported by spiking BM with prostate cancer cell line cells. Using micromanipulation, we isolated on average 4 EpCAM+ cells from a positive BM sample. In total, we isolated 225 single cells or clusters from M0 samples, 51 single cells from HD samples, and 16 single cells or clusters from M1 samples. After quality analysis of amplified cDNA, we analysed the expression of EPCAM, KRT8, KRT18, KRT19, KRT14, KRT6a, KRT5, KLK3 (PSA), MAGEA2, MAGEA4, PTPRC (CD45), CD33, CD34, CD19, GYPC, SLC4A1 (band 3), and HBA2 in 124 M0 samples, 28 HD samples, and 10 M1 samples, using PCR. We detected in significantly higher number of EpCAM+ cells from M0 CaP patients transcripts for EPCAM (p=0.001), KRT8 (p=0.01) and KRT18 (p=0.05), compared to cells from HD. On the other hand, transcripts for e.g. PTPRC or HBA2 were detected at similar frequency. Overall, no transcript or group of transcripts could reliably distinguish DCC from hematopoietic cells. The analysis of genomic aberrations, currently the gold standard for identification of DCC, revealed that cell harbouring genomic aberrations, and hence true DCC, often express hematopoietic markers, while not expressing e.g. KLK3. These results point to an unexpected plasticity of epithelial cancer cells in BM, suggesting their adaptation to the specific environment, and question common transcriptional criteria to identify DCC or circulating tumour cells.
Identification and molecular characterization of different subpopulations of EpCAM-positive single disseminated cancer cells in prostate cancer

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The detection of early disseminated cancer cells (DCC) in bone marrow (BM) of prostate cancer (CaP) patients without distant metastasis is associated with poor prognosis. Patients that may benefit from aggressive therapies are difficult to identify and this leads to considerable overtreatment. DCC comprise the founder cells of later arising metastasis, and therefore they are direct targets for adjuvant therapies. DCC are extremely rare and can be detected in BM by using antibodies against epithelial markers, such as cytokeratins (CKs) and epithelial cell adhesion molecule (EpCAM). We have previously observed that CK⁺ DCCs in BM detected in samples taken before surgery are associated with poor survival of CaP patients. In contrast, detection of persisting CK⁺ cells in BM samples up to 8 years after surgery had no impact on the survival of patients, indicating that prostate cancer can become a chronic, non-progressive disease. This result prompted us to search for cells that persist after surgery, need not express cytokeratins, and are associated with metastatic progression. We therefore searched for EpCAM⁺ cells in a new cohort of 220 CaP patients. This cohort contained patients with M0-stage disease, biochemical relapse (BR), and M1-stage disease. While the number of different stage patients (M0, BR, and M1) with CK⁺ cells in BM was constant, the number of patients with EpCAM⁺ cells doubled between M0 and BR, and between BR and M1. Then, we directly compared the prognostic impact of CK⁺ and EpCAM⁺ DCCs detected in BM samples taken months to years after surgery. Strikingly, while CK⁺ cells were, as previously shown, not informative about progression, the detection of EpCAM⁺ DCCs after surgery was associated with poor survival. Furthermore, double staining against both epithelial markers identified different subsets of DCCs (CK⁺/EpCAM⁻, CK⁺/EpCAM⁺, and CK⁻/EpCAM⁺). This finding was confirmed by EPCAM, KRT8, and KRT18 transcript analysis in another cohort of 105 CaP patients. The analysis of single DCCs from BM by comparative genomic hybridization demonstrated that both CK⁺ and EpCAM⁺ cells are tumour cells. Interestingly, not only that EpCAM⁺ cells harboured significantly more genomic aberrations than CK⁺ cells when analysed across different disease stages (M0, biochemical relapse, and M1), but they were also defined by characteristic genetic changes. Furthermore, we analysed prostate-derived cell lines, in which expression of EpCAM was up- or down-regulated, for their sphere generating potential and migration. These results suggest that cells with activated EpCAM signalling display more aggressive phenotype. Overall, these results indicate an unexpected diversity of early disseminated cancer cells displaying different phenotypes and genotypes, and divergent metastatic potential in patients.
A41 Integrin-independent migration and metastasis formation of fibrosarcoma and melanoma tumor cells

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Cell migration is the crucial mechanism underlying invasion of cancer cells into healthy tissue, followed by intravasation and distant metastasis formation. Surface receptors of the integrin family have been implicated in mediating cancer invasion and metastasis based on their key role in mediating cell-matrix adhesions and cell migration in vitro. In addition, integrins enhance survival signaling and thus are considered as targets for anti-cancer therapy to inhibit tumor dissemination and progression.

To assess the role of integrins for survival, invasion and metastasis formation of HT1080 fibrosarcoma and MV3 melanoma cells, xenografts of these cells were orthotopically implanted into nude mice and monitored in vivo by a dorsal skin-fold chamber model combined with intravital multiphoton microscopy. Without interference these tumors display predominantly collective invasion along pre-existing tissue structures which means that multicellular strands of physically and functionally connected cells extend from the primary tumor and migrate along and between collagen fibers, muscle strands, blood vessels and nerve bundles.

HT1080 and MV3 cells are positive for β1 and β3 integrin. By targeting both molecules through stable shRNA-mediated knockdown combined with integrin blocking antibodies 4B4 (β1) and 17E6 (β3), >99% reduction of integrin availability is reached. After dual integrin interference, both HT1080 and MV3 xenografts underwent spontaneous regression, confirming a role for integrins in survival signaling. However, despite partial tumor regression, invasive tumor cell subsets survived and migrated independent of integrins by diminished collective and enhanced single-cell migration. Furthermore, besides local invasion, dual integrin targeting enhanced distant metastasis formation to the lungs by increasing the number of micro-metastases while the amount of macro-metastasis was decreased. These data suggest that integrins are dispensable for local invasion and distant metastasis, but required for rapid growth and cell survival.

In conclusion, therapeutic targeting of β1 and β3 integrins may enhance, rather than diminish, metastatic dissemination.
Expression of chemokine CXCL14/BRAK whether in melanoma cells or in non-tumor cells in their environment suppresses metastasis to the lung

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The multistep nature of cancer is associated with gradual increases in tumor size, disorganization and malignancy such as occurs in invasion and metastasis. And each step occurs depending on the malfunction of different genes. Earlier, we produced 3 lines of transgenic mice (Tg) expressing CXCL14/BRAK gene under the control of beta-actin promoter and CMV enhancer. Significant differences in tumor volumes of B16 melanoma or Lewis lung carcinoma (LLC) cell transplants were observed between wild type C57BL/6 (Wt) and Tg mice. Here we tested the effect of overexpression of this chemokine on the metastatic rate of the cells by using these Tg mice. Using experimental metastasis model, we found that the number of metastatic nodules of melanoma cells and LLC cells was significantly ($P<0.001$) lower in the Tg mice than in the Wt ones; and that this suppressed metastasis was attenuated by the injection of NK cell-depleting anti-asialo GM1 antibody or anti- NK 1.1 antibody.

Then in order to investigate the effect of CXCL14/BRAK expressed in melanoma cells on the metastatic rate of the cells, we produced melanoma cells expressing this chemokine under the control of doxycycline (B16Tet/On BRAK) and provided C57BL/6 mice with 5% sucrose or 5% sucrose containing 2 mg/mL of doxycycline in their drinking water. After injection of the melanoma cells via a tail vein, the number of metastatic nodules on the lungs was significantly ($P<0.01$) lower in the mice fed with doxycycline. When the melanoma cells were injected into SCID mice deficient in T, B, and NKT cells, the number of metastatic nodules increased approximately 4 fold compared with that in the Wt animals; but still the number of nodules in the animals that has not been treated with doxycycline was twice that of those treated with it. On the other hand, when the cells were injected into NOG mice, which are deficient all lymphocytes including NK cells, the number of metastatic nodules increased 10 fold over that in the Wt mice; and the numbers of metastatic nodules in the NOG mice lungs were the same regardless of doxycycline administration. These data indicate that expression of CXCL14/BRAK molecule in the melanoma cells suppressed the metastatic rates NK-cell dependently.

These data indicate that expression of CXCL14/BRAK, whether in the tumor cells or in cells in their micro environmental cells, suppressed the metastatic rates of the tumor cells and this effect was at least partly dependent on the NK cell activity.
A43  Insulin-like Growth Factor II mRNA-Binding Protein 3 Expression Promotes Tumor Cell Migration through NF-κB pathway and Predicts Poor Prognosis in localized Clear Cell Renal Cell Carcinoma

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Purpose: Insulin like growth factor 2 mRNA binding protein 3 (IMP3) is a novel oncoprotein. Patients with localized RCC that expressed IMP3 had a shorter survival than those without IMP3 expression. However, the role of IMP3 in RCC progression was poorly understood. In this study, we aim to validate the prognostic significance of IMP3 of patients with localized CCRCC (clear cell RCC) in a large cohort and explore the mechanism of IMP3 regulating the cell migration and invasion.

Methods: The IMP3 expression was analyzed by IHC in tissue microarray including 469 clinically localized CCRCC and correlated with the pathologic parameters and survival. The Caki-1 cell line stably overexpressing IMP3 and Achn cell line with knockdown of IMP3 were established and their migration abilities were measured by transwell assay. RNA sequencing was used to identify different expressing genes in IMP3-Caki-1 cell and the regulating function of NF-κB signaling pathway in cell migration was investigated.

Results: IMP3 was expressed in 73 (16%) of 469 tumors. Expression was associated with higher nuclear grade, higher T stage, necrosis and sarcomatoid differentiation (P < 0.001). IMP3-positive tumors were associated with shorter recurrence-free survival and overall survival. Multivariable analysis validated IMP3 as an independent prognostic factor. IMP3 could promote cell motility, migration and invasion in Caki-1 cells. In comparison, knockdown of IMP3 in Achn cells inhibited cell migration by RNA interference. Overexpression of IMP3 significantly increased the expression of genes involved in NF-κB pathway. The ability of IMP3 improving migration of RCC cells was inhibited by NF-κB pathway inhibitor.

Conclusions: We validate that IMP3 was an independent prognostic marker for localized CCRCC. IMP3 can promote the migration and invasion of RCC cells and may function through NF-κB pathway.
A44 Investigating tumor heterogeneity with pRainBow: a novel cell barcoding system

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It is becoming intensely apparent that tumor heterogeneity is a confounding factor to proper diagnosis, staging, and treatment of cancer patients. Concomitantly, tumor metastasis is an inherently heterogeneous process. For example, it is well known that metastatic lesions often respond differently to therapy than the primary tumor. However, the biological mechanisms underlying this type of heterogeneous response are unknown. Therefore, it is important to develop novel technologies that will help us investigate and understand the biological processes driving heterogeneity and how they affect patient care. Here we present a novel fluorescent protein barcoding system that will aid significantly in the study of tumor heterogeneity. Fluorescent or luminescent imaging of tumors in animal models is a critical element in dynamic and longitudinal monitoring of tumor burden, tumor growth, and metastatic dissemination. Currently, most imaging strategies are limited to one or two different colors per animal. To improve utility of current imaging technologies, we have developed a library of fluorescent and luminescent tracking vectors designed to permit in vivo multiplexing through fluorescent barcoding. Our lentiviral-based vectors express firefly luciferase and one of six spectrally unique fluorescent proteins that cover the entire fluorescent protein color palette from blue to far-red. The unique combination of luciferase and fluorescent labeling allows us to monitor tumor growth by luciferase activity and distinguish individual cell populations by their fluorescent label. We have stably labeled bone-derived MDA-MB-231 cells with these tracking vectors by viral transduction. Using these individually colored cell lines, we have demonstrated that the six FPs are uniquely identifiable by spectral un-mixing with the Maestro Q Imaging System and by flow cytometry. We have demonstrated the utility of this barcoding system in the study of tumor heterogeneity through a mouse model of skeletal metastasis. Using the barcoded MDA-MD-231 cells, we determined that dissemination to the bone is a cooperative event, whereby multiple tumor cells are able to seed and proliferate in a single bone. Additionally, we have investigated the “homing” ability of tumor cells selected from a specific metastatic site (eg. the lung and bone) and found that, indeed, these cells tend to seed in the organ from which they were isolated. Finally, we intend to expand the number of unique barcodes in this tool by multiplexing the fluorescent proteins as well as integrating unique epitope tags in order to enhance its utility in studying tumor heterogeneity.
A45 Specific miRNA signatures characterize different metastatic sites in clear cell renal cell carcinoma

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Background: MiRNAs are regulators of gene expression in tumorigenesis and progression. To identify miRNAs associated with metastasis miRNA expression in distant metastases was compared to primary clear cell renal cell carcinoma (ccRCC).

Material and methods: Total RNA of 27 primary ccRCC samples and 25 distant metastases (lung, bone and brain) was isolated from formalin-fixed paraffin-embedded (FFPE) samples Microarray analyses were performed for a global miRNA expression profiling. Results were validated by qPCR. For miRNA target identification a ccRCC cell line (786-O) was transfected with miRNAs differently expressed in metastatic primary tumors and metastases.

Results: We identified 9 miRNAs (including miR-30c) with a similar expression in metastatic primary ccRCC and distant metastases from different metastatic sites compared to non-metastatic primary ccRCC. 11 miRNAs (including miR-30c-2-3p) were differently expressed in distant metastases compared to primary ccRCC. Furthermore, each metastatic site is characterized by a specific miRNAs (including miR-199b and miR-375). Results were verified on selected miRNAs using qPCR. Target identification for metastasis associated miRNAs is ongoing.

Discussion: These data suggest that miRNAs play an important role in metastatic processes of ccRCC. Furthermore, our results regarding different metastatic sites suggest two important statements. Specific miRNAs characterize distant metastases in general. On the other hand, miRNA expression is associated with specific conditions at different metastatic sites. Thus, the data presented in this study give the base for a better understanding of the involvement of miRNAs as regulators of metastasis which opens new possibilities for new targeted therapy options.
A46  The tumour-promoting receptor tyrosine kinase, EphB4, regulates expression of integrin β8 in prostate cancer cells

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EphB4 is a receptor tyrosine kinase that is over-expressed in 66% of prostate cancers and has been shown to play vital roles in cell migration and invasion in a variety of epithelial tumours. It is recognized as a potentially important therapeutic target. Little is known about the intrinsic pathways by which EphB4 promotes tumour progression. A key objective of this study was to define the molecular networks that the EphB4 receptor influences in prostate cancer. To examine this, we employed transient knock down of EphB4 in LNCaP cells followed by cDNA microarray analysis, coupled with bioinformatic approaches, to identify genes regulated by loss of EphB4. Validation experiments were carried out on selected target genes using quantitative real-time PCR and western immunoblotting to confirm their differential, EphB4-mediated expression. We also examined these genes following over-expression of EphB4. The microarray analysis revealed that 260 genes were upregulated and 300 were down-regulated when EphB4 was knocked down (by 70 %) in LNCaP prostate cancer cells. Gene ontology analysis showed the process of cell adhesion as being most significantly influenced. Several integrins appeared to be deregulated, but Integrin β8 (ITGB8) was the top hit with a 29-fold down-regulation in EphB4 siRNA treated cells, compared to control cells (treated with a scrambled, control siRNA). Strikingly, as might be expected, over-expression of EphB4 led to a simultaneous increase in ITGB8 expression. The integrin receptors play an essential role in the communication between cells and the extracellular matrix, influencing adhesion, migration and invasion of cancer cells. Whilst several members of the integrin family have been a focus in prostate cancer, nothing is known about the role of ITGB8 in this disease. Analysis using the Oncomine clinical cohort database revealed that ITGB8 and EphB4 are both highly expressed in prostatic intraepithelial neoplasms (PIN) with decreasing expression in prostate carcinomas and basal expression in metastases, suggesting roles in the early stages of prostate cancer progression. In conclusion, we have discovered that EphB4 regulates ITGB8 expression and that they are concomitantly expressed in prostate cancer and that both are highly expressed in PIN. This suggests that EphB4 and ITGB8 could be involved in the onset of prostate cancer and targeting these two proteins synergistically may impact on prostate cancer progression.
Tumor-targeting *Salmonella typhimurium* A1-R inhibits breast cancer bone metastasis

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In this study, we measured the efficacy of *Salmonella typhimurium* A1-R (A1-R) on breast cancer bone metastasis in nude mice. We established an early-stage bone-metastasis model in nude mice by cardiac injection and a late-stage model by injection into the intramedullary cavity of the tibia using MDA-MB-435 human breast cancer cells (PLoS One 5, e9712, 2010). Late and early stage bone metastasis were treated with A1-R. Fluorescence imaging was performed to visualize the metastatic bone lesions. In the early-stage bone-metastasis model, A1-R significantly improved metastasis-free survival. In the advanced-stage bone-metastasis model, A1-R significantly inhibited the growth of the metastatic lesions. These data indicated that A1-R is useful to prevent and inhibit the growth of bone-metastatic breast cancer.
The combination of *Salmonella typhimurium* A1-R and anti-VEGF therapy inhibits patient-derived orthotopic xenograft (PDOX) pancreatic cancer

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The aim of this study was to examine the efficacy of *Salmonella typhimurium* A1-R (A1-R) treatment on VEGF-positive human pancreatic cancer in a patient derived orthotopic xenograft (PDOX) model in combination with anti-VEGF therapy. A VEGF-positive pancreatic cancer PDOX and VEGF-positive human pancreatic cancer cell line (MiaPaCa-2-GFP) were orthotopically implanted in nude mice. The nude mice were treated in the following groups: (1) gemcitabine (GEM) (80 mg/kg, ip, weekly, 4 weeks); (2) GEM (80 mg/kg, ip, weekly, 4 weeks) + bevacizumab (Bev) (5 mg/kg, ip, twice a week, 4 weeks); (3) GEM (80 mg/kg, ip, weekly, 2 weeks) + Bev (5 mg/kg, ip, twice a week, 2 weeks) → A1-R (1.5x10\(^8\) CFU/body, ip, weekly, 2 weeks); and (4) saline (vehicle/control, ip, weekly, 4 weeks). The tumor weight of each group in the PDOX model was as follows: (1) GEM; 263.1 ± 129.1 mg, (2) GEM/Bev; 65.9 ± 41.9 mg, (3) GEM/Bev→A1-R; 21.9 ± 6.2 mg and (4) Control; 998.8 ± 377.7 mg. GEM/Bev→A1-R significantly reduced tumor weight compared to GEM/Bev treatment in the PDOX model (p=0.029). The mean tumor weight of each group in the MiaPaCa-2-GFP model was as follows: (1) GEM; 775.9 ± 273.8 mg; (2) GEM/Bev; 413.5 ± 108.3 mg; (3) GEM/Bev→A1-R; 257.5 ± 57.1 mg; and (4) Control; 2655.4 ± 583.9 mg. GEM/Bev→A1-R significantly reduced tumor weight compared to GEM/Bev treatment in the MiaPaCa-2-GFP model (p=0.022). These results demonstrate that A1-R is effective on pancreatic cancer in combination with anti-VEGF, including the PDOX model, indicating the clinical potential of this combination.
The advantages of patient derived orthotopic xenograft (PDOX) of cervical cancer compared to the PDX model

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In this study, we compared the use of a patient derived orthotopic xenograft (PDOX) compared to a subcutaneously-grown patient cervical cancer in nude mice (PDX model). A PDX (F1) from the patient with a metastatic HER-2-positive cervical cancer in nude mice was made. We implanted to the small fragments of F1 PDX to 30 nude mice subcutaneously (PDX) and 30 nude mice orthotopically (PDOX) to examine the efficacy of various drugs for this tumor. Six weeks after implantation, the mice in each model were randomized to 5 groups and treated in the following groups: (1) trastuzumab (20 mg/kg, ip, weekly, 5 weeks) + lapatinib (100 mg/kg, orally, daily, 5 weeks); (2) entinostat (5 mg/kg, orally, daily, 5 weeks); (3) trastuzumab (20 mg/kg, ip, weekly, 5 weeks) + lapatinib (100 mg/kg, orally, daily, 5 weeks) + entinostat (5 mg/kg, orally, daily, 5 weeks); (4) carboplatin (30 mg/kg, ip, weekly, 5 weeks); and (5) saline (vehicle/control, ip, weekly, 5 weeks). The relative tumor volume, compared to time zero, of each group in the PDX model was as follows: (1) 1.23 ± 0.50; (2) 3.07 ± 1.79; (3) 0.38 ± 0.18; (4) 0.39 ± 0.23; (5) 10.23 ± 2.91. All regimens had significant efficacy compared to the untreated control group. The trastuzumab/lapatinib/entinostat combination and carboplatin were the most effective regimens in the subcutaneous model. The relative primary tumor volume of each group in the PDOX model was as follows: (1) 0.70 ± 0.23; (2) 3.25 ± 1.16; (3) 0.54 ± 0.34; (4) 0.48 ± 0.39; (5) 4.45 ± 1.09. The metastatic tumor weight of each group in PDOX model was as follows: (1) 5.7 ± 9.8 mg; (2) 20.1 ± 25.1 mg; (3) 4.7 ± 8.1 mg; (4) 0 ± 0 mg; (5) 112.0 ± 32.5 mg. All regimens had significant efficacy on the primary tumors compared to the control group except for the entinostat-alone group. However, entinostat alone significantly reduced the metastatic tumor weight compared to the control. The trastuzumab/lapatinib/entinostat combination and carboplatin were the most effective regimens on both primary and metastatic tumors in the PDOX model. However, only the PDOX model metastasized and could be used to discover the anti-metastatic activity of entinostat alone which was not effective on the primary tumor.
A50  Fluorescence-guided surgery and neoadjuvant chemotherapy on a pancreatic cancer patient derived orthotopic xenograft (PDOX)

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The aim of this study is to determine the efficacy of fluorescence-guided surgery (FGS) in combination with neoadjuvant chemotherapy (NAC) on a pancreatic cancer patient derived orthotopic xenograft (PDOX). We established a PDOX model from a patient with metastatic CA19-9-positive pancreatic cancer. Forty nude mice were implanted with tumor using surgical orthotopic implantation (SOI) on the nude mouse pancreas. Four weeks after implantation, the mice with tumor were randomized into 4 treatment groups: (1) bright-light surgery (BLS) only; (2) FGS; (3) NAC-BLS; (4) NAC-FGS. Gemcitabine (80 mg/kg, ip, weekly, 3 weeks) was used for NAC. Seven weeks after implantation, BLS was performed on all tumor-bearing mice. A monoclonal anti-human CA19-9 antibody conjugated with Dylight 650 was delivered to tumor-bearing mice in the FGS groups as a single intravenous dose 24 hours before BLS. Post-operatively, the surgical resection bed of mice to be treated with FGS were imaged with the OV100 fluorescence imaging system to detect fluorescent residual tumors. The residual tumors after BLS in the FGS groups were then resected under fluorescence navigation. The average resected tumor weight of each group was as follows: (1) BLS, 188.5 ± 50.0 mg; (2) FGS, 278.0 ± 111.4 mg; (3) NAC-BLS, 84.5 ± 48.7 mg; (4) NAC-FGS, 141.8 ± 48.9 mg. The average resected tumor weight of NAC-treated mice was significantly less than the non-NAC-treated mice (113.1 ± 57.0 mg and 235.6 ± 94.3 mg, respectively; p>0.001). The average resected tumor weight of the FGS-treated mice was significantly larger than the BLS-treated mice (213.4 ± 107.1 mg and 136.5 ± 73.8 mg, respectively; p=0.016). Eight weeks after resection. The recurrence rate of each group was as follows: (1) BLS, 9 / 9 (100%); (2) FGS, 4 / 8 (50%); (3) NAC-BLS, 6 / 9 (66.7%); (4) NAC-FGS, 2 / 8 (25%). The recurrence rate of FGS-treated mice was significantly lower than BLS-treated mice (6 /16; 37.5% and 15 / 18; 83.3%, respectively; p=0.012), whereas there was no significant difference between FGS and NAC-FGS-treated mice regarding recurrence (p=0.608). There was no significant difference in the total recurrence rate between NAC and non-NAC-treated mice (p=0.157). However NAC significantly reduced peritoneal recurrence (from 29 % in the non-NAC group to 0%, p=0.044). The results indicate that NAC in combination with FGS can reduce or even eliminate peritoneal recurrence of pancreatic cancer.
A51  The first patient derived orthotopic xenograft (PDOX) model of patient cervical cancer

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A tumor resected from a patient with metastatic HER-2-positive cervical cancer was implanted in 8 NOD/SCID mice (F1; NOD/SCID-sc) and 10 nude mice subcutaneously (F1; nude-sc), and 8 nude mice orthotopically (F1; nude-soi). We examined the tumor-take rate of each model 8 weeks after implantation. Tumors grew in 3 out of 3 mice in F1; NOD/SCID-sc (100 %); 7 out of 10 mice in F1; nude-sc (70 %); and 6 out of 8 mice in F1; nude-soi (75 %). Metastasis (peritoneal dissemination, liver metastasis, lung metastasis or lymph node metastasis) were detected in 4 mice in F1; (nude-soi) (50 %). We found all growing tumors including the metastatic tumors had histological structures similar to the original tumor and were stained by anti-human HER-2 antibody similar to the resected tumor. These results suggest that the cervical cancer the PDOX nude mouse model recapitulates the biological behaviors of the original tumor.
A52 Targeting tumor-educated macrophages by zoledronic acid inhibits proliferation and metastasis of human pancreatic cancer in nude mice

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Tumor-educated macrophages (EMφ) from tumor-bearing mice promote tumor growth and metastasis. The aim of the present study was to determine the efficacy of zoledronic acid (ZA) on EMφ. EMφ and naïve macrophase (NMφ) were compared for their ability to enhance tumor progression. ZA killed both EMφ and NMφ in vitro. We then demonstrated that EMφ promoted tumor growth and metastasis in an orthotopic mouse model of a human pancreatic cancer cell line: ZA reduced the tumor growth (p = 0.006) and metastasis (p = 0.025) promoted by EMφ. We then examined the efficacy of ZA for pancreatic cancer in the patient-derived orthotopic xenograft (PDOX) model. The combination of gemcitabine (GEM) and ZA reduced tumor weight (p = 0.016) and tumor growth (p = 0.005) compared to GEM alone in the PDOX model. ZA alone reduced metastasis (p = 0.009). These results suggest that ZA inhibits the proliferation and the metastasis of human pancreatic cancer by targeting EMφ.
**A53** *Salmonella typhimurium* A1-R inhibits experimental breast cancer brain metastasis

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The efficacy of *Salmonella typhimurium* A1-R (A1-R) on breast cancer brain metastases was evaluated. A high brain-metastatic variants of murine 4T1 breast cancer cells expressing red fluorescent protein (RFP) were injected into the left ventricle of transgenic nude mice expressing nestin-driven green fluorescent protein (ND-GFP) in nascent blood vessels. At various time points, the tumors and vasculature in the brain were imaged by confocal fluorescence microscopy. Eighty percent of the cells that reached the brain extravasated and grew perivascularly. Twenty percent of the cells, proliferated within the brain vasculature. Mice treated with *Salmonella typhimurium* A1-R (A1-R) had significant inhibition of brain metastasis and increased survival (*p*<0.05).
A54  *Salmonella typhimurium* A1-R inhibits breast cancer metastasis induced by primary tumor resection

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The aim of this study was to determine if tumor-targeting *Salmonella typhimurium* A1-R (A1-R) inhibited metastasis induced by tumor resection. Fifty female BALB/c mice were inoculated in the mammary fat pad with metastatic murine mammary adenocarcinoma (4T1-RFP \(3\times10^5\)/mouse), and divided into five groups. Control group (n = 10) with no treatment; surgery group (n = 10) in which the primary tumor was completely resected at day 21 after cell inoculation; A1-R (n = 10) in which the primary tumor was treated with A1-R alone at day 10 after cell inoculation; postoperative-administration of A1-R (n = 10) in which A1-R was administrated only after the primary tumor was resected; perioperative administration of A1-R (n = 10) in which A1-R was administrated before and after the primary tumor was resected. Metastatic tumor burden was assessed by quantitative fluorescence imaging of metastasis. Excision of the primary tumor was associated with increased systemic metastatic burden, and postoperative metastases proliferated rapidly (\(p < 0.05\)). A1-R alone inhibited metastasis compared to untreated control and surgery only (\(p < 0.05\)). Post-operative A1-R treatment significantly inhibited surgically-induced metastases (\(p < 0.05\)) but had no significant difference from A1-R alone with no surgery (\(P > 0.05\)). Perioperative administration of A1-R showed the best efficacy compared to other treatments in inhibiting primary-tumor resection-induced metastasis (\(p < 0.05\)) and prolonged survival (\(p < 0.05\)).
A55 Targeting Neuropilin-1 to inhibit prostate cancer metastasis and therapy resistance

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Objective: Recent reports provide evidence that the epithelial-to-mesenchymal transition (EMT) plays a key role in PCa metastasis and therapy resistance. We have recently identified the cell surface receptor, Neuropilin-1 (NRP1) to be increased during EMT and this study aims to determine whether the inhibition of NRP1 will be a feasible therapeutic strategy for blocking PCa metastasis and therapy resistance.

Methods: qRT-PCR and western blotting was used to determine the expression levels of NRP1 in metastatic PCa cell lines. NRP1 expression in clinical samples was assessed using immunohistochemistry and bioinformatic analysis of multiple independent patient cohorts. RNAi approaches were used to assess the functional role of NRP1 in cell migration and invasion.

Results: NRP1 expression is elevated in metastatic PCa cells. \textit{In vitro} studies have revealed the suppression of endogenous NRP1 levels to significantly inhibit the migratory and invasive behaviour of metastatic PCa cells. Importantly, NRP1 is increased in metastatic and therapy resistant clinical PCa samples and high NRP1 levels to be associated with shorter time to tumour relapse and shorter overall survival.

Conclusion: These results will provide the preclinical data necessary to rationalise the use of anti-NRP1 directed therapies for clinical use in PCa patients. Pharmaceutical companies currently have antibody and small molecule inhibitors under early development as anticancer therapeutics. This study will pave the way for larger scale preclinical and clinical trials in the PCa setting, with the ultimate goal of accelerating the translation of these therapeutics into the clinic for PCa patients.
A56 Gene amplification of $ACTN4$ is a predictor of cancer metastasis

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Actinin-4 is an essential protein for forming the cell protrusions related to cell movements and is closely associated with cancer invasion and metastasis (1, 2). Immunohistochemical analysis revealed that overexpression of actinin-4 correlated significantly with poor prognoses for breast (1), ovarian (3), and lung cancer (4). Gene amplification of $ACTN4$, the gene producing actinin-4, is responsible for overexpression of actinin-4 in some patients. We recently reported that gene amplification of $ACTN4$ offers a very good biomarker for identifying patients with poor prognosis for stage-I adenocarcinoma of the lung (n=290) using fluorescent in situ hybridization. The 5-year survival rate of patients with stage-I adenocarcinoma of the lung without gene amplification of $ACTN4$ was 95%, significantly longer than the 5-year survival rate of 58% for patients with gene amplification of $ACTN4$ (4). We also identified copy number increase (CNI) for $ACTN4$ as a predictive biomarker for the metastatic potential of salivary grand carcinoma (5), ovarian cancer (6), and pancreatic cancer (7). Actinin-4 is biologically involved in cancer metastasis, and gene amplification of $ACTN4$ offers an indicator of metastatic potential in cancer.

References
A57  EGF-induced recycling of the cancer promoting protein CDCP1

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Many cancers are dependent on inappropriate activation of epidermal growth factor receptor (EGFR) and drugs targeting this receptor can improve patient survival, although benefits are generally short-lived. We reveal a novel mechanism linking EGFR and the membrane spanning, cancer promoting protein CDCP1. Under basal conditions cell surface CDCP1 constitutively internalizes and undergoes palmitoylation-dependent degradation by a mechanism in which it is palmitoylated on at least one of its four cytoplasmic cysteines. This mechanism is functional in vivo as CDCP1 is elevated and palmitoylated in high grade serous ovarian tumors. Interestingly, activation of the EGFR system with EGF inhibits proteasome-mediated, palmitoylation-dependent degradation of CDCP1, promoting recycling of CDCP1 to the cell surface where it is available to mediate its pro-cancer effects. We also show that mechanisms inducing relocalization of CDCP1 to the cell surface, including disruption of its palmitoylation and EGF treatment, promote cell migration. Our data provide the first evidence that the EGFR system can function to increase the lifespan of a protein and also promote its recycling to the cell surface. This information may be useful for understanding mechanisms of resistance to EGFR therapies and assist in the design of treatments for EGFR-dependent cancers.
Molecular mechanisms of early cancer cell dissemination in breast cancer

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Accumulating evidence indicates that tumor cells spread to distant sites much earlier than previously thought. The analysis of the BALB-neuT transgenic mouse model of breast cancer revealed that the relative number of disseminating cancer cells is highest at early stages of tumorigenesis. To unravel the mechanisms of early dissemination, we performed a stage-dependent analysis of gene expression patterns in BALB-neuT mice. At the stage of atypical ductal hyperplasia (ADH) gene expression profiles differed substantially from advanced tumors and lung metastases. We identified an ADH-specific gene expression signature and found that among the several hundred differentially expressed genes, many of them are regulated by steroid hormones. We investigated whether this expression pattern might be linked to the propensity to disseminate. We established a surrogate signature for the ADH-specific gene expression pattern comprising five genes and tracked the regulating role of the hormones in vitro and in vivo. Our results indicate that steroid hormones act indirectly on mammary stem and progenitor cells to induce dissemination and metastasis and those hormone nuclear receptors are down-regulated in advanced tumors. Thereby, large tumors become less capable of disseminating metastatic cells.
A59  LOX and TEM8, a new relationship affecting tumor and metastatic growth?

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There are marked similarities between the effects of LOX (lysyl oxidase) and TEM8 (tumor endothelial marker 8) on tumor growth, invasion and angiogenesis. LOX is an amine oxidase secreted from tumor cells that catalyzes the crosslinking of collagens and elastin in the extracellular matrix. We have previously shown that LOX expression is clinically correlated with metastasis and decreased survival. Consistently, we find that LOX expression is low in non-metastatic cancer cells and high in metastatic cancer cells, and that manipulating LOX expression/activity can modulate metastatic ability. Tumor-derived LOX promotes the secretion of vascular endothelial growth factor, which stimulates tumor angiogenesis both in colorectal and breast cancer models. LOX also plays a role in tumor progression, metastasis, and pre-metastatic niche formation through its ability to modulate matrix stiffness. Intriguingly, microarray analysis showed a significant increase in TEM8 mRNA levels in breast cancer cells seeded on collagen modified by LOX. Thus, we speculate that another possible mechanism of LOX mediated tumor angiogenesis and growth progression is through TEM8.

TEM8 is an integrin-like cell-surface receptor originally identified being specific to tumor endothelial cells in colorectal cancer (CRC) blood vessels. Further investigation showed upregulated TEM8 expression in tumor vessels of various tumor types, as well as in certain tumor cells. We observe an increase in TEM8 expression in endothelial cells in response to conditioned medium containing LOX. We also observe a strong association between the expression of TEM8 and LOX in tumors from a CRC mouse model. We further note elevated TEM8 mRNA levels in a metastatic breast cancer cell line compared with its non-metastatic counterpart cell line, with high and low LOX expression levels respectively. Preliminary data show that a humanized anti-TEM8-toxin-conjugated antibody has preclinical effectiveness in a CRC model in mice.

Based on our initial data we hypothesize that LOX mediates cancer progression through TEM8. Further, that combined targeting of TEM8 and LOX may inhibit primary and metastatic tumor growth.
Metastatic prostate cancer cells incline to initiate metastasis colonization and inflammation in the lung

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Hormone-refractory and metastatic prostate cancer (PCa) both result in therapeutic failure. Developing PCa animal model is critical to understand the cancer progression and to identify useful therapeutic targets. Here, we established orthotopic xenograft base on androgen-independent 22Rv1 cell line in nude mouse model and propagated the spontaneous metastases. The same procedure was repeated thrice to evolve highly metastatic cells, 22Rv1-LN3. In experimental metastasis, 22Rv1-LN3 colonized and grew tumors in the lymph nodes, lung and brain through tail vein injection but 22Rv1 cell failed to colonize in these organs. Besides, intracardiac injection of 22Rv1-LN3 also caused bone metastasis. In the model, we found 22Rv1-LN3 cell have higher protease activity (e.g. matriptase and MMP9) along with lower level of protease inhibitors, (e.g. HAI-2, a matriptase inhibitor and TIMP2/TIMP3). Transcriptional levels of these genes causally contribute to the in vitro invasion activity and in vivo tumorigenicity. Given the differential capacity to seed different tissues, we further analyze the stem cell property by tumorsphere assay and in vivo transplantation with a reduced cell number. Sphere-formation rate in 22Rv1-LN3 cells was 8% compared to only 0.1% in 22Rv1 cells. Tumorigenicity of 22Rv1-LN3 cells in orthotopic transplantation was 100% (8/8) compared to 20% (2/10) in 22Rv1 cells. Moreover, primary tumor of 22Rv1-LN3 cells developed spontaneous metastases in lymph nodes and lung at 8-week, whereas 22Rv1 cells hardly metastasized even at 14-week. As analyzed by qRT-PCR, primary 22Rv1-LN3 tumor vs. 22Rv1-Luc2 induced higher levels of ARG1 and NOS2 expression in lung tissue, which indicates the lung inflammation and proclivity for metastatic niche formation. In conclusion, this PCa progression model not only elevated the malignant properties of cancer cells but also inflamed the tumor microenvironment.
A61 Integrating SNPs, epigenetics and transcriptomics to better understand the inherited predisposition to breast cancer metastasis

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Breast cancer is the most frequent cancer and the second leading cause of cancer mortality in women. The vast majority of breast cancer mortality is due to metastatic disease since the primary tumor can be relatively easily surgically resected. More comprehensive understanding of biology of metastasis is therefore clearly warranted to unveil novel metastasis-associated molecules and cellular processes that might be targeted for clinical intervention. Previously we demonstrated that like cancer incidence, metastatic progression has a significant inherited component. Using mouse complex trait mapping strategies we have been isolating metastasis susceptibility genes from backcross analysis. However, studies suggest that many of these QTL peaks are the result of the contribution of multiple genes, suggesting that causal genes are not being identified. To increase our ability to detect causal genes we have implemented a new integrated strategy. Whole genome sequencing of appropriate high or low metastatic mouse strains is performed to identify variants, which are then filtered for those within DNAse hypersensitive sites (DHS), based on the hypothesis that most inherited phenotypes are due to expression differences rather than missense variants. The genes associated with polymorphic DHS are then screened through mouse and human tumor expression databases to identify those genes associated with development of metastatic disease. This strategy was able to identify genes previously associated with metastatic breast cancer in both mouse and humans and new genes have also been validated. The results further suggest that inherited variation in cellular mediated immune response may be an important contributor to metastatic disease.
A62 Combined targeting of mTOR and c-MET significantly inhibits epithelioid sarcoma cell growth

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Epithelioid sarcoma (EpS) is a relatively rare high-grade soft tissue sarcoma with mixed epithelial and mesenchymal phenotype. EpS is characterized by loss of integrase interactor 1 (INI-1) expression. The clinical course of EpS is usually characterized by local recurrence and distant metastasis to lymph nodes and lungs, but the effective chemotherapy has not been established until now. Therefore, novel therapeutic approaches against EpS are critically needed.

Recently, INI-1-deficient tumor cells were reported to exhibit persistent activation of AKT signaling. We also found loss of INI-1 expression and constitutive activation of the AKT/mTOR pathway in two human EpS cell lines, Asra-EPS and VAESBJ. AKT activation has been proposed to be a predictor of response to mTOR inhibitors, and thus we examined the antitumor effects of an mTOR inhibitor RAD001 (Novartis) on these EpS cell lines. RAD001 inhibited EpS cell proliferation, but reactivation of AKT and ERK occurred after RAD001 treatment.

An intrinsic resistance to mTOR inhibitors has been reported to be caused by receptor tyrosine kinase (RTK)-dependent AKT reactivation due to a release of negative feedback inhibition. We sought activated RTKs in EpS and found that c-MET was the most highly activated RTK in both Asra-EPS and VAESBJ cells. A c-MET inhibitor INC280 (Novartis) suppressed EpS cell growth by blocking the activation of c-MET and its downstream molecules such as AKT and ERK, suggesting that one mechanism for the activation of AKT and ERK in EpS was through the c-MET signaling pathway.

RAD001-induced reactivation of AKT and ERK was decreased by c-MET inhibition with anti-c-MET siRNAs or INC280 treatment, providing a rational for combining RAD001 with INC280 to treat EpS patients.

Indeed, combination of RAD001 and INC280 exerted superior antitumor effects on the growth of EpS cell lines both in vitro and in vivo. Therefore, our preclinical data suggest that combined targeting of mTOR and c-MET can be a novel and effective strategy for the treatment of human EpS lacking systemic treatment.
A63 Dynamic analysis of lung metastasis by mouse osteosarcoma

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Osteosarcoma (OS) is the most common malignant bone tumor and the prognosis depends on pulmonary metastases, which arise from multi-step progression of malignant tumors. We herein aimed to clarify the critical step of pulmonary metastasis using the syngeneic mouse spontaneous highly metastatic OS LM8 and parental Dunn cell lines, to identify new candidate molecules to suppress pulmonary metastasis. We first investigated the chronological detection of circulating tumor cells (CTCs) from mice with either cell line. LM8 CTCs appeared faster, at a higher rate and with a greater number compared to Dunn CTCs. Cultured cells from CTCs of LM8 showed higher proliferative ability than cells from the primary site in suspension culture, which mimicked the environment of the bloodstream for CTCs. The proliferative ability of LM8 cells was also higher than that of Dunn cells in 3D collagen culture with low stiffness (~150 Pa; close to conditions in the lung). We next focused on the extravasation step. LM8 showed higher migration ability compared to Dunn with transendothelial migration assay. We also found a disruption in endothelial barrier function throughout co-culture with LM8 using time-lapse imaging. In addition, LM8 secreted high levels of vascular endothelial growth factor (VEGF), while VEGF signal inhibition with a small molecule tyrosine kinase inhibitor (pazopanib) decreased disruption of the vascular barrier and transendothelial migration of LM8. Finally, daily oral administration of pazopanib reduced the rate and size of pulmonary metastasis in vivo. Collectively, these results show anti-VEGF therapy as a candidate for pulmonary metastasis of OS.
Slug induces invasive behavior, but not epithelial-mesenchymal transition, in chronic hypoxia LNCaP human prostate cancer cells.

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Introduction: Tumor hypoxia is a common feature of any cancer including prostate cancer and is associated with tumor progression. We had previously reported that chronic hypoxia accelerated cell migration and invasion. We investigated whether chronic hypoxia induced epithelial-mesenchymal transition (EMT) in prostate cancer cells.

Methods: The human prostate cancer cell line LNCaP was cultured under normoxia (21% O2), acute hypoxia (1% O2 for 48 hours), or chronic hypoxia (1% O2 for 1 month and 6 months). We investigated expressions of key EMT molecules at the level of mRNA and protein in each LNCaP.

Results: The expression of Slug (SNAI2) which is known as a transcriptional factor and a key molecule of EMT, was increased and Zonula Occuludens (ZO)-1 which is known as a tight junction protein, was decreased. But the other EMT markers were not significantly changed. Knockdown of Slug inhibited cell migration and invasion in chronic hypoxia LNCaP cells.

Conclusions: These results suggested that chronic hypoxia is not associated with EMT in prostate cancer cells. But Slug and ZO-1 might be associated with prostate cancer progression.
A65 Can surgery prior to first circulating tumor cell (CTC) appearance cure cancer? : Experiments based on CTC-mouse model

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Purpose: Theoretically, curative resection of primary tumor before tumor dissemination can cure cancer. Previously, we developed circulating tumor cells (CTC) allograft mouse model. Using CTC-mouse model, we tested the hypothesis that curative resection prior to CTC appearance results in repression of tumor metastasis.

Methods: CTC-mouse model was developed as fo llow. 4T1 GFP-Luc2-transfected-mouse mammary tumor cell lines were injected into mammary fat of female BALB/c mice. Primary tumor volume (mm³) was measured with digital caliper. Metastasis was evaluated with IVIS® in vivo imaging system. 15μl blood were collected and GFP positive-CTCs were counted by flow cytometry.

Results: One hundred BALB/c mice were used to develop CTC-mouse model. In most cases, CTC-mouse died 6 weeks later orthotropic transplantation of 4T1 cell lines. At 5 weeks, means of tumor volumes and CTC counts were 1739±1034 mm³ and 94±87/15ul, respectively. We found a certain correlation (Spearman’s value 0.731) between CTC count and tumor volume. We found CTC in every case when tumor volume reached at 140 mm³. We defined the optimal tumor volume enabling to identify CTC as 140mm³. Then, we removed primary tumor when tumor volume was measured around 140mm³. Among 20 mice with primary tumor removal, 4 mice showed no tumor recurrence and paucity of CTC for 5 weeks after operation and 5 mice for 3 weeks. Mice with regrowth of tumor died due to tumor progression despite of primary tumor removal.

Conclusions: Our data implies that R0 resection prior to CTC occurrence can inhibit tumor progression and metastasis.
A66  Serum Amyloid A and its role in tumor metastasis

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The acute phase protein serum amyloid A (SAA) is implicated in inflammatory diseases, amyloidosis and tumor progression. It accumulates in the blood during inflammation, and for many human cancers increased circulatory levels are associated with reduced patient survival. In previous studies together with Mariam Grigorian, we found that SAA expression in an experimental animal model of breast cancer was sufficient to promote widespread metastasis, which was accompanied by massive immune cell infiltration into the tumors. Furthermore, SAA increased motility and invasion of tumor cells and stimulated expression of pro-inflammatory genes.$^1$ Now, we aim to provide further proof of principle demonstrations that SAA is a valid therapeutic target, and to develop ways of inhibiting its activity. Ongoing work in the lab focuses on the establishment of experimental mouse models to study the role of SAA in metastasis. First experiments with syngeneic tumor models have revealed that different SAA isoforms are expressed in tumors in vivo. Gain and loss of function studies with these tumor models will allow us to determine the effect of tumor-produced SAA on metastasis. The production of recombinant SAA proteins is ongoing, which will then be injected in experimental animals, allowing us to evaluate systemic effects of circulating SAA on metastasis. To complement this approach, we plan in addition to increase systemic levels of SAA artificially using SAA adenoviruses. Our ultimate aim is to establish SAA inhibitors (antibodies, peptides) as a novel therapeutic approach.


* Equal contribution
A67  Snail, a possible switch for Epigenetic alteration in the development of ovarian cancer

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Snail, a key regulator of epithelial-mesenchymal transition (EMT), plays an important role in development of ovarian cancer. Our previous works have confirmed that Snail expression correlated with the stage of ovarian cancer. To further investigate the underlying mechanisms of Snail function in ovarian cancers, we performed the Reverse Phase Protein Assay (RPPA) using the Snail induced SKOV3 cell lines. It revealed that Dnmt1(DNA (cytosine-5)-methyltransferase 1), was extremely up-regulated in Snail induced cells compared to the non-induced cells and led to the subsequent alteration of global DNA methylation status. A follow-up MeDIP-chip analysis revealed that the up-regulation of DNMT1 by Snail negatively regulated apoptotic signaling pathway and positively regulated cell growth and proliferation signaling pathway. The expression of 5-methylcytosine(5’mc) and 5-hydroxymethylcytosine(5’hmC) in the snail inducible cells were also examined, which showed that 5’mc turned up when snail were induced while 5’hmC weakened obviously in the same scenario. Overall, Our data indicated a new role of Snail in epigenetic process in the development of ovarian cancer and uncovered that Snail alter the epigenetic status, through regulating the expression of DNMT1, which maybe the important mechanism in the regulation of proliferation and metastasis of ovarian carcinoma.
A68 The inflammatory cytokine OSM induces breast cancer metastasis by promoting early stages of metastasis

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The propensity of primary breast cancer cells to metastasize has been partially explained by high levels of endogenous inflammation. However, it is not fully understood how inflammatory mediators, expressed either by the primary breast cancer cells or cells of the microenvironment, specifically contribute to metastasis. Our lab has demonstrated that oncostatin M (OSM), an interleukin-6 (IL-6)-family inflammatory cytokine, has a role in the development of early metastatic properties distinct from IL-6 and other family members. We have shown by tissue microarray of human breast samples that OSM is expressed at highest levels in the precancerous epithelial cells of ductal carcinoma in situ (DCIS), suggesting a role for autocrine-produced OSM in the development of invasive capacity. We investigated the effect of OSM on early stages of breast cancer metastasis utilizing an orthotopic 4T1.2 mouse model. These experiments showed that reduced OSM expression in 4T1.2 cells (4T1.2-shOSM) decreased lung metastasis and circulating tumor cell numbers and increased animal survival post-primary tumor resection. However, these effects were not seen if the early stages of metastasis were bypassed by injecting the 4T1.2-shOSM cells directly into the systemic circulation. Furthermore, peri-tumoral injections of human OSM in a human MDA-MB-231 xenograft system resulted in an increase in the number of circulating tumor cells as well as spontaneous metastasis to lung. Finally, we demonstrated a mechanism for OSM in early stage metastatic potential in vitro by showing OSM-induced tumor cell detachment, migration, invasion, and IL-6 expression. These studies suggest that autocrine and paracrine OSM in the tumor microenvironment acts as a potent initiator of the early stages of metastasis. Therefore, modification of OSM levels in the tumor inflammatory environment could be a highly effective therapeutic strategy for halting the metastasis of breast cancer.

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A69  The metastasis suppressor NME1 suppresses melanoma cell motility through alterations in integrin repertoire and focal adhesion dynamics

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Expression of the metastasis suppressor NME1 in melanoma is associated with reduced cellular motility and invasion \textit{in vitro} and metastasis \textit{in vivo}, but the underlying molecular mechanisms are not fully understood. Consistent with its metastasis suppressor function, we observe that NME1 expression dramatically reduces random, single cell motility in multiple NME-deficient, metastatic melanoma cell lines (e.g. 1205LU and M14). Forced NME1 expression induced a morphological transformation in 2-dimensional cultures from a bipolar, spindle shape with multiple filopodia to a spread out, polygonal appearance. This change was associated with generation of a network of thick intracellular stress fibers that terminated at large focal adhesions and foci of fibronectin fibrils. Time-lapse video TIRF (total internal reflection fluorescence) imaging of paxillin-labeled focal adhesions (FAs) in the metastatic cell lines revealed rapid FA turnover at both the leading and trailing edges of cells, with new FAs appearing continuously within newly-formed pseudopodia at the leading edge. In contrast, NME1 induced larger and more stable FAs that migrated from the edges towards the cell interior and were continuously replaced at all edges, resulting in a “treadmilling” effect with little or no net cell movement. In parallel, NME1 induced a nearly total switch in cell surface expression of the fast-recycling $\alpha_4\beta_1$ integrin to the slower recycling $\alpha_v\beta_3$ form. Inhibition of integrin $\beta1$ (ITGB1) expression by NME1 was shown to be exerted at the level of mRNA expression, and not via regulation of dynamin-mediated endocytosis or protein stability (proteosomal or lysosomal degradation). Downregulation of ITGB1 expression by NME1 was ablated by two point mutations (E\textsubscript{5}A and K\textsubscript{12}Q) that we showed previously to disrupt metastasis suppressor function. In addition, an inverse correlation was observed between the expression of NME1 and ITGB1 mRNAs across a large cohort of melanoma biopsies, strongly suggesting this regulatory axis is operative in the human disease. The inverse correlation between NME1 and ITGB1 expression was also a strong predictor of both prolonged distant disease-free and overall survival in patients with the basal-like subtype of breast carcinoma. Together, these observations strongly suggest the metastasis suppressor function of NME1 is driven to a significant degree by inhibition of ITGB1 mRNA and protein expression, resulting in reduced FA recycling and suppression of cell motility.
A70  Neutrophilic granule proteins (Ngp) is a SPARC target gene to be reduced.

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SPARC, that is a matricellular protein, positively contributes cancer malignancy such as metastasis. We hypothesize that SPARC modulates gene expression in lung and affects pulmonary metastasis. In this study, we determine gene expression profiling in lung of SPARC knockout mouse in three kinds of mouse strain (MF1, C3H, and C57Bl/6) by using DNA microarray. Commonly up-regulated gene numbers in lung of SPARC knockout mouse between 2 strains: MF1 & C57Bl/6, 78 genes; MF1 & C3H, 62 genes; C57Bl/6 & C3H, 6 genes and among 3 strains, 4 genes including Ngp (neutrophilic granule proteins), Saa3 (serum amyloid 3) and Camp (encoding antimicrobial peptide LL-37). Commonly down-regulated gene numbers in lung of SPARC knockout mouse between 2 strains: MF1 & C57Bl/6, 10 genes; MF1 & C3H, 6 genes; C57Bl/6 & C3H, 0 gene and among 3 strains: 0 gene. These results suggested that SPARC modulates some gene expression but majority of their modulation was depending on mouse strain. On the other hand, SPARC siRNA, targeting both mRNA variant 1 (nt 887 to 905) and the variant 2 (nt 884 to 902) showed upregulation of Ngp but not obviously for Saa3 and Camp in both B16-F1 and B16-BL6 cells. Our data suggested that Ngp is a SPARC target gene to be reduced. Because number of metastasized foci in SPARC knockout mouse was lower than in wild type mouse, elevation of Ngp may be involved in prevention of metastasis in SPARC knockout mouse.
A71 High systemic VEGF-C level lead to a specific myeloid cell accumulation in the pre-metastatic lung in a syngeneic rat breast cancer model

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Vascular endothelial growth factor (VEGF)-C and its receptor VEGFR-3 are key regulators of lymphangiogenesis, the outgrowth of new lymphatic vessels from pre-existing ones. VEGF-C is expressed by various metastatic tumors and can induce lymphangiogenesis both peritumorally as well as in tumor-draining lymph nodes. Thus, VEGF-C induced high lymphatic vessel density increases the probability for invasive tumor cells to enter the vasculature, thereby contributing to metastasis formation. We have reported recently that local intradermal injection of VEGF-C is able to promote metastasis to the regional lymph nodes, but not to distant organs in the syngeneic non-metastatic breast cancer model NM081. This observation indicates that the stimulation of lymphangiogenesis in the tumor periphery is not sufficient to promote metastases to distant organs. However we showed that VEGF-C expressing NM081 tumors metastasize to regional lymph nodes as well as to the lung. Furthermore we found high serum levels of VEGF-C in breast cancer patients and in the syngeneic highly metastatic breast cancer model MT450. Taken together these data indicate that VEGF-C can not only act locally but also systemically to promote metastasis, but the underlying mechanism is still unknown.

Previous studies revealed that tumors can prepare organs for the arrival of disseminating tumor cells by establishing so called pre-metastatic niches, future sites of metastasis in target organs. Primary tumors can produce TDSFs (tumor-derived secreted factors) which lead to the recruitment of CD11b⁺ bone marrow-derived myeloid cells that contribute to the formation of pre-metastatic niches. Importantly, we could show that VEGF-C is able to act on the bone marrow compartment.

This work aims at investigating the role of systemic VEGF-C in the formation of metastatic niches. First results show that systemically applied recombinant VEGF-C indeed led to a significant and specific accumulation of CD11b⁺ myeloid cells in the lungs of experimental rats, whereas the number of CD11b⁺ cells in liver and spleen remained unchanged. In the lung, myeloid cell clusters can be found in the periphery but not close proximity of terminal bronchioles, an area where lung metastases occurs frequently. Furthermore, in a syngeneic VEGF-C secreting rat breast cancer model, clusters of myeloid cells occurred in the pre-metastatic lung. These data suggest that systemic VEGF-C induces CD11b⁺ myeloid cell accumulation in the lung prior to the arrival of disseminating tumor cells, thereby contributing to the formation of a pre-metastatic niche. Ongoing work aims at the further characterization of the myeloid cell population and assessing whether VEGF-C-induced CD11b⁺ cell accumulation is sufficient to promote metastasis in a non-metastatic syngeneic rat breast cancer model.
A72 Omental macrophages promote ovarian cancer metastatic colonization

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Metastatic ovarian cancer remains an urgent clinical problem. The homing and invasion of cancer cells into the omental adipose tissue, the preferred site of ovarian cancer metastasis, is a rate-limiting step in disease progression. Improving patient outcomes requires a mechanistic understanding of how cancer cells colonize the omentum and ultimately give rise to widespread peritoneal metastases. Unlike other peritoneal adipose, omental adipose contains functional immune aggregates known as milky spots, which contain macrophages, B, T, natural killer (NK), and stromal cells within capillary nests. In addition to their role in peritoneal homeostasis and immune defense, our data shows that milky spots play an active role in ovarian cancer metastatic colonization. We hypothesize that omental microenvironment plays a crucial role in instructing the ovarian cancer cells to achieve an aggressive phenotype that enables widespread metastases.

Well-established in vitro migration assays and in vivo experimental metastasis assays were used to answer the following questions: Does cancer cell localization depend upon the immune composition of the milky spots? Do cancer cells utilize the native milky spot microenvironment, or does it undergo remodeling during colonization? Answers to these questions will be the foundation for mechanism-based studies aimed at identifying ovarian cancer-omental interactions that can be targeted therapeutically.

Human (SKOV3ip.1, CaOV3, HeyA8), and murine (ID8) ovarian cancer cells rapidly localized to omental milky spots and not adipose lacking milky spots. Use of genetic models (C57BL/6, Athymic Nude, Beige Nude, Rag1−/−, Igh6−/− mice) ruled out a requirement of B, T, and NK cells in ovarian cancer cell homing to milky spots suggesting a critical role for macrophages in this process. Quantitative data found no difference in the incidence of cancer cell foci in the omentum across these strains. Our recent data shows that depletion of omental adipose tissue macrophages (ATM) abrogates cancer cell colonization of milky spots. In vitro assays have found that omental adipose-conditioned media causes 75% more cell migration and contains a higher concentration of macrophage-secreted cytokines than media conditioned by adipose lacking milky spots. Importantly, our data shows that factors secreted by omental ATMs have an enhanced ability to promote ovarian cancer migration compared to factors secreted by other peritoneal ATMs.

We have shown that omental milky spots are required for cancer colonization of peritoneal adipose. Further, we have identified omental macrophages as the key mediators of colonization. Future studies are focused on understanding the cancer cell-macrophage interactions that can be targeted therapeutically to disrupt metastatic growth and extend disease-free survival.

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A73 3D matrix regulates stemness properties in mouse melanoma cells by Id1 and Id3 induction

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Cancer stem cells (CSCs) are thought to drive tumor growth and metastasis through their unique tumor initiating properties. Tumor cells that disseminate from the original primary tumor but which remain in a dormant state in the body would therefore be expected to (re)acquire CSC properties in order to initiate metastatic outgrowth. Remodelling of the extracellular matrix (ECM) in the microenvironment of dormant cells has been implicated in the transition from dormancy to proliferative growth. Consistently, tumor initiation in vivo is strongly increased by coinjecting tumor cells with artificial ECM components, suggesting a critical role for the extracellular matrix in determining CSC properties. Gene expression profiling of tumor cells in a 3D ECM environment showed that ECM components induce the expression of Id1 and Id3, transcriptional regulators that govern stem cell identity and prevent premature differentiation of normal stem cells. Moreover, Id1 and Id3 have been demonstrated to play a key role in the ability of tumor cells to initiate primary and metastatic growth. Mechanistically, we have found that a variety of 3D ECM environments promote autocrine BMP signalling, probably by acting as a mechanical barrier that inhibits diffusion of the BMP molecules, thereby increasing local BMP concentrations and leading to increased expression of Id1 and Id3. Although Id1 an Id3 have been implicated in determining the stemness properties of tumor cells, Id overexpression in the melanoma cells did not promote chemoresistance to doxorubicin or cisplatin. However, it did confer a survival and proliferation advantage as assessed by colony formation assays. Compounds targeting increased Id expression or function may therefore prove useful for effective cancer treatment.
A74  Breast cancer metastasis to gynaecological organs: impacting young women diagnosed with a luminal tumour subtype

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Approximately 90% of breast cancer deaths are due to metastatic dissemination. The most common sites of breast cancer metastasis include the lung, liver and bone, however breast tumours can also spread to sites such as the gynaecological organs. Metastasis to gynaecological organs accounts for 7-10% of all gynaecological malignancies and as they are hard to diagnose, patients can be asymptomatic for many years and thus metastases tend to be wide spread when diagnosed and hence overall survival is poor. In order to understand the molecular phenotype of breast tumours that spread to the gynaecological organs and the biological mechanisms influencing this pattern of organotropism, we have begun analyzing clinical details, tumour pathology features and immunohistochemical phenotyping data (ER, PR, HER2, CK5/6, CK14, EGFR, p53, Ki67, AR, E-cadherin, β-catenin and p120-catenin) from a series of 54 breast cancer patients who developed metastasis to gynaecological organs.

The average age of patients at diagnosis of their primary tumour was 49 years (median 47 years, range 30-79), which is younger than the average age of primary tumour diagnosis in Australia (60 years), while the average time to metastasis was 6 years (range 0-20 years). Forty three percent of tumours were invasive lobular carcinoma. This represents an enrichment for ILC spreading to gynaecological tissues given that ILC normally accounts for 5-15% of breast cancer diagnoses. Seventy-four percent of patients developed metastasis to additional sites; most commonly, peritoneum/omentum (19.1%) followed by gastrointestinal organs (9.3%). Interestingly, the common sites of breast cancer metastasis were rarely involved: bone = 4.2%; lung = 1.4%; liver = 1.9%; and brain = 1.4%. Immunohistochemical staining of tissue microarrays showed that the primary tumours were largely ER positive (88.1%), PR positive (75.9%), HER2 negative (96.4%) and basal marker (CK5/6, EGFR and CK14) negative (100%). Concordance of immunophenotype has been assessed for ER and PR and it was found that expression remained unchanged during metastatic progression in 75.7% of cases for ER and 52.1% for PR.

In conclusion we found that patients with gynaecological metastasis represent a young patient cohort, with a significant burden of metastatic disease, most notably to other abdominal cavity organs. The primary tumour and metastases are almost always ER and PR positive and lack markers of traditionally aggressive phenotypes (HER2 and basal markers). The stable expression of ER during progression suggests a potential role for hormone regulation in dissemination to these particular organs. Further work will help uncover the mechanism of spread to these particular sites.
A75 Overcoming radiotherapy resistance and plasticity of non-adherent stem-like and adherent prostate cancer cells by targeting Erk and Akt signaling

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Fractionated ionizing radiation combined with surgery or hormone therapy represent the first-choice treatment for medium to high risk localized prostate carcinoma. In prostate cancer, the failure of radiotherapy is often caused by radioresistance and further dissemination of escaping cells. To investigate radioresistance-associated phenotype, we exposed four metastasis-derived human prostate cancer cell lines (DU145, PC-3, LNCaP and 22RV1) to clinically relevant daily fractions of ionizing radiation (35 doses of 2 Gy) resulting in generation of two surviving populations: adherent senescent-like cells expressing common senescence-associated markers and non-adherent anoikis-resistant stem cell-like cells with active Notch signaling and expression of stem cell markers CD133, Oct-4, Sox2, and Nanog. While the radioresistant adherent cells were capable of resuming proliferation shortly after the end of irradiation, the non-adherent cells started to proliferate only after their reattachment occurring several days after the irradiation-driven loss of adhesion. Like the parental non-irradiated cells, radioresistant reattachment DU145 cells retained tumorigenic potential after injection to immunocompromised mice. The radiation-induced loss of adhesion was dependent on expression of Snail, as siRNA/shRNA-mediated knock-down of Snail prevented cell detachment. On the other hand, survival of the non-adherent cells required active Erk signaling, as chemical inhibition of Erk1/2 by a MEK-selective inhibitor or Erk1/2 downregulation by siRNAs resulted in anoikis-mediated death in the non-adherent cell fraction. Notably, whereas combined inhibition of Erk and PI3K-Akt signaling triggered cell death in the non-adherent cell fraction and blocked proliferation of the adherent population of the prostate cancer cells, such combined treatment had only marginal if any impact on growth of control normal human diploid cells. Importantly, irradiated re-adherent cells exhibit less senescent-like colonies in clonogenic cell survival assay and enhanced anoikis-resistant survival upon reirradiation pointing to the acquired radioresistance. These results contribute to better understanding of radiation-induced stress response and heterogeneity of human metastatic prostate cancer cells, document treatment-induced plasticity and phenotypically distinct cell subsets, and suggest the way to exploit their differential sensitivity to radiosensitizing drugs in overcoming radioresistance.

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A76 Identification of exosomal proteins involved in breast cancer cell invasion

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Invasion and metastasis are continuing therapeutic challenges and common causes of death for patients with breast cancer. Cancer cell-derived exosomes containing proteins are linked to the disease pathogenesis in various cancers. Here, we investigated whether a protein in exosomes secreted from a representative invasive breast cancer cell line, MDA-MB-231, contribute to invasion through exosome-mediated mechanism in breast cancer. As a result, the Del-1 on exosomes is sufficient for enhancement of cancer cell invasion and for acceleration of lung metastasis in mouse models. This invasion is most likely mediated via the integrin-FAK signaling cascade in cancer cells. However, these effects are significantly suppressed when the Del-1 is inactivated, providing evidence for a critical role of Del-1 in development of cancer. Furthermore, targeting Del-1 on exosomes may lead to a new therapeutic option for treatment of breast cancer.
A77 Cyclooxygenase-2 signaling promotes prostate cancer cell invasion and the activation of matriptase

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Chronic inflammation has been proposed to participate in prostate cancer development and progression. Several studies have indicated that cyclooxygenase-2 (COX-2), a key enzyme to generate prostaglandins during inflammation, is overexpressed in primary prostate cancer with metastatic potential. However, the molecular mechanism how COX-2 promotes prostate cancer cell invasion and metastasis is not well understood. In this study, we investigated the role of COX-2 in prostate cancer cell invasion and delineated the molecular mechanism in which COX-2 signaling increased prostate cancer cell invasion. Using overexpression and knockdown approaches, the results showed that COX-2 overexpression could enhance prostate cancer PC3 cell invasion and up-regulate membrane-anchored serine protease matriptase expression, while COX-2 silencing decreased the cancer cell invasion and the levels of activated matriptase. Moreover, prostaglandin E2 (PGE2), a major product of COX-2, was able to induce prostate cancer cell invasion and matriptase activation. Furthermore, NSAID sulindac sulfide and celebrex could significantly suppress prostate cancer cell invasion and down-regulate matriptase expression. Together, the data indicate that COX-2 is involved in promoting prostate cancer cell invasion and matriptase activation, suggesting that suppression of COX-2 signaling may be a strategy to reduce prostate cancer progression.
A78  Alterations of the hepatic microenvironment modulate the phenotype and behavior of disseminated pancreatic ductal epithelial cells

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most common lethal cancer entities in western countries and has an overall 5-year survival rate of only 7 %. Most PDAC patients are diagnosed in a metastasized stage, which excludes curative treatment and accounts for the high fatality rate of the disease. The predominant site of metastasis in PDAC is the liver. However, little is known about the influence of the hepatic microenvironment on disseminated pancreatic tumor cells. Recent mouse studies suggest that cells from PDAC precursor lesions can leave the pancreas and disseminate prior to the histological manifestation of PDAC. In this study, an indirect coculture system encompassing the precancerous human pancreatic ductal epithelial (HPDE) cell line or the tumorigenic PDAC cell line Panc-1 and the hepatic stromal cell lines M1-4HSC and M-HT was used to examine the influence of the hepatic microenvironment on the phenotype and behavior of precancerous HPDE cells. Whereas the hepatic stellate cell line M1-4HSC was used to simulate a physiological hepatic microenvironment, its transdifferentiated myofibroblastoid variant M-HT simulated an inflamed situation.

We showed that coculture with M1-4HSC cells significantly reduced the proliferation of HPDE and Panc1 cells and resulted in an enhanced phospho-p38- and p21 activity besides an unaltered phospho-ERK expression. Importantly, indirect coculture with M1-4HSC cells induced senescence in both cell lines, as determined by their enlarged morphology, the absence of the proliferation marker Ki-67 and a senescence-associated β-galactosidase (SABG) activity. Concomitantly, the coculture with M-HT cells resulted in a diminished phospho-p38 activity and induced proliferation of both cell lines.

Elevated levels of interleukin (IL)-6 and IL-12 during M1-4HSC coculture were identified as part of the senescence-associated secretory phenotype (SASP) in M1-4HSC cocultures whereas M-HT cocultures showed enhanced levels of VEGF and SDF-1α. Preliminary data from immunohistochemical stainings of liver sections from an endogenous PDAC model indicate an increased proliferation of pancreatic tumor cells predominantly within micrometastasis surrounded by a myofibroblast-rich stroma whereas single PDAC cells were mainly quiescent and detected in liver tissue areas lacking myofibroblasts.

This study suggests that the hepatic microenvironment has a marked influence on the phenotype and behavior of premalignant and malignant pancreatic cells. Along with an established link between inflammation and PDAC progression, our findings suggest that an inflamed hepatic microenvironment is more permissive for the proliferation while a physiological hepatic microenvironment hampers the propagation of disseminated preneoplastic and neoplastic pancreatic cells.

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NME1 Mediates a Switch in Beta Integrin Subunits that Correlates with Prolonged Patient Survival

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Expression of the metastasis suppressor NME1 in melanoma is associated with reduced cellular motility and invasion in vitro and metastasis in vivo, but the molecular mechanisms underlying this activity are not completely understood. Herein we report a novel mechanism through which NME1 suppresses motility by modulating focal adhesion dynamics via regulation of integrin β1. As predicted, forced expression of NME1 strongly suppressed random cell motility in the melanoma cell lines 1205LU and MDA-MB-435s/M14, both of which exhibit high metastatic potential in athymic nude mice. The decrease in cell motility caused by NME1 expression was found to occur through dramatically reduced turnover of focal adhesions. Interestingly, over-expression of NME1 resulted in a switch in from predominantly fast recycling α4β1 integrins to slower recycling αvβ3 integrins. Contrary to its regulation of other cell surface receptors, the inhibition of integrin β1 by NME1 was found to occur at the RNA level rather than through control of dynamin mediated endocytosis. The inhibition of integrin β1 required both the 3-5’ exonuclease and nucleoside diphosphate kinase (NDPK) activities of NME1, which are also required for its metastasis suppressor activities in vivo. Furthermore, an inverse correlation was observed between NME1 and integrin β1 mRNA in a large cohort of primary melanoma biopsies. No correlation in mRNA levels was found in metastatic melanoma biopsies, which may be due to downregulation of NME1 protein expression rather than mRNA in metastases. The inverse correlation of NME1 and integrin β1 RNA was also a strong predictor of prolonged distant disease free and overall survival in patients with the basal-like subtype of breast carcinoma. Together, these data strongly suggest NME1 prevents metastasis of human melanoma and some types of breast cancers by inhibiting integrin β1 expression to reduce recycling of focal adhesions and, ultimately suppress of cell motility.
A80 Does neoadjuvant radiotherapy and the timing of surgery modify metastatic dissemination?

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Neoadjuvant radiotherapy (RT) is used in many treatments and aims at improving tumor local control and patient overall survival. RT schedule and the timing of surgery are mostly empirical based on clinical experiences. Tumor microenvironment plays a crucial role in tumor growth and metastatic dissemination. Modifying this environment with radiotherapy may influence the tumor phenotype and the formation of metastases. With an original pre-clinical model of neoadjuvant RT, we study the impact of different RT schedules on tumor inflammatory microenvironment and on tumor dissemination according to the timing of surgery.

Human mammary tumors, implanted into the flank of SCID mice, were irradiated with different neoadjuvant RT schedules (i.e. 5x2Gy and 2x5Gy). We surgically removed carefully tumors 4 or 11 days after the end of RT and kept the mice alive during 5 weeks for metastatic growth. Then we sacrificed the mice and searched for lung metastases thanks to human Ki-67 immunohistochemical staining.

After 2x5Gy, the size and the number of lung metastases were smaller when surgery was performed at 11 days after the end of RT, compared to 4 days. Inversely, in the 5x2Gy schedule, applying surgery at 4 days protected the mice against lung metastases compared to surgery at 11 days. These results suggest that the timing of surgery and RT schedules are both important factors that influence the formation of metastases.

We first investigated several pathways well known to contribute to cancer progression and metastatic dissemination such as hypoxia, vessel density, proliferation and necrosis. No obvious difference was seen between the 4 experimental groups. We next focused our interest on the inflammation, a process known to play a crucial role in cancer progression and, which could be influenced by RT.

Thanks to computer assisted quantification performed on F4/80 stained tumor sections, we observed a significant difference in tumor macrophage infiltration according to the timing of surgery. Macrophage infiltration appears to increase at day 11 compared to day 4 after 2x5Gy RT and to decrease at day 11 compared to day 4 after 5x2Gy RT. In sharp contrast, no difference was seen according to the RT schedule.

RNA level of iNOS (a marker M1 macrophage subtype) and Arginase 1 (a marker of M2) by real time PCR appeared correlated with the occurrence of metastases. Thus, we studied the different subsets of tumor-associated macrophages and inflammatory cells by FACS analyses. Although many differences were observed between the experimental groups, none of them explained the difference in lung metastases occurrence.

We developed a powerful pre-clinical model to study the impact of neoadjuvant RT schedules and the timing of surgery on tumor microenvironment and metastatic dissemination. This model reveals the importance of the tumor inflammatory microenvironment at the time of surgery for tumor dissemination. Further investigations are needed to highlight the precise role of inflammatory microenvironment in the metastatic phenotype.
A81  Tumor-suppressor mir-449a regulates MEK1-ERK1/2-AP1 through an auto-regulatory feedback circuit in lung cancer*

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Metastasis is the most deleterious clinical consequence arising in lung cancer and it causes the majority of cancer-related mortality. Emerging evidence reveals that miRNAs can regulate the tumor metastasis by acting on multiple signaling pathway. In this study, we found that miR-449a is significantly downregulated in highly metastatic lung cancer cell line and distant metastatic lymphoid using microarray analysis and real-time PCR experiments. Then, both gain-of-function and loss-of-function experiments showed that increased miR-449a expression significantly suppressed the migration and invasion of highly metastatic lung cancer cell line L9981, whereas decreased miR-449a expression markedly promoted the migration and invasion of low metastatic lung cancer cell line NL9980, and in vivo metastasis experiment also revealed that overexpression of miR-449a dramatically suppressed lung cancer metastasis and tumorigenesis, suggesting that miR449a may be a tumor metastasis suppressor. Moreover, the MAP2K1 oncogene, which is a mitogen-activated protein (MAP) kinase kinase 1 (MEK1), participating in the MEK1/ERK1/2/c-jun signal pathway cascade that play critical role in cancer metastasis, was identified as a direct target of miR-449a. Further studies indicated that overexpression of miR449a inhibits the expression of p-MEK1, p-ERK1/2, c-jun and p-c-jun and results in p-ERK1/2, p-c-jun dephosphorylation, whereas inhibition of miR-449a enhanced the expression of p-MEK1, p-ERK1/2, c-jun, p-c-jun, but the upregulation effect can partially reversed by MEK1 inhibitor. Finally, we found that miR-449a is a direct transcriptional target of AP-1(c-jun/c-fos dimer), and miR-449a expression is epigenetically repressed through histone H3 Lys27, 9 trimethylation in lung cancer cells. Thus the present study provides insight into a novel negative feedback mechanism through which miR-449a inhibits lung cancer metastasis. Targeting this miR-449a/MEK1/AP1/miR449a negative feedback would be helpful as a therapeutic approach to impede lung cancer metastasis.

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**A82 NIFK overexpression is associated with poor survival of lung cancer and promotes tumor invasion through repression of casein kinase 1α to activate TCF/β-catenin signaling**

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Lung cancer metastasis is the pivotal cause which results in cancer-related deaths. It is urgently needed to identify novel prognostic markers that can predict metastatic risk, and useful therapeutic targets that can reduce mortality. There is emerging evidence supporting Ki67 as a biomarker for prediction of poor prognosis and recurrence in various types of cancer including lung cancer. However, little is known about the role of its binding protein nucleolar protein interacting with the FHA domain of pKi-67 (NIFK). We explore novel roles of NIFK during lung cancer progression. NIFK was discovered highly expressed in lung cancer specimens and correlated with poor survival of patients. In addition, overexpression of NIFK enhanced cell migration, invasion and metastasis in A549 and PC13 lung cancer cells, whereas knockdown of NIFK in H661 and H1299 cells reduced cell migration and *in vivo* metastasis. Microarray analysis via knowledge-based Ingenuity Pathway Analysis (IPA) and MetaCore identified the downregulation of the suppressor for pro-metastatic TCF4/β-catenin signaling, casein kinase 1α (CK1α), by NIFK. CK1α was complementary upregulated after NIFK knockdown. Further repression of CK1α expression in cells of NIFK knockdown restored TCF4/β-catenin transcriptional activity, cell migration and *in vivo* metastasis. Runx1 and SRY were further identified as novel transcription factors responsible for CK1α expression, and were negatively regulated by NIFK. Taken together, our results demonstrate the prognostic value of NIFK, and reveal its crucial role in promoting lung cancer metastasis through Runx1 and SRY-mediated CK1α repression, leading to the TCF4/β-catenin signaling activation in lung cancer progression.
Macrophages regulate early metastatic dissemination in a model of ErbB2+ breast cancer.

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Metastases originate from tumor cells that disseminate from the primary site through a process aided by macrophages. This was proposed to occur only during late invasive stages of cancer. However, the presence of tumor cells in the bone marrow of patients with ductal carcinoma in situ (DCIS), a pre-invasive form of breast cancer, argues that these events can take place earlier. The mechanisms driving this process are poorly understood. Our lab showed that in the MMTV-ErbB2 breast cancer model, inhibition of p38 signaling accelerated disease progression and enhances early dissemination. Using this same model and knowing that macrophages are key for mammary gland development and remodeling, we investigated whether these inflammatory cells might actively participate in early dissemination. We now show that F4/80+ macrophages associate with mammary ducts in the periphery of the myo-epithelial layer but are never inside the ducts in wild type mouse mammary glands. We found that macrophages are recruited into epithelial layers of pre-malignant lesions of MMTV-ErbB2 mice. This is dependent on the up regulation of cytokines in mammary epithelial cells induced by the amplification of the ErbB2 oncogene and loss of p38 activity. This event showed a high correlation with enhanced dissemination of ErbB2+CK8/18+ mammary epithelial cells (MECs) detected in circulation or the bone marrow. Sites where macrophages had invaded the ducts showed disruption of the myo-epithelium and reduced E-Cadherin expression in MECs. Depletion of macrophages from pre-malignant MMTV-ErbB2 mice prevented early dissemination. Importantly, DCIS samples from patients frequently contained intra-epithelial macrophages. These data suggest that macrophages may actively aid early dissemination from pre-malignant tissue, but only in the context of p38 inhibition and ErbB2 activation. Further, in areas of macrophage infiltration, luminal ductal cells may down regulate E-cadherin, which could foster cell migration/invasion. Our data in human DCIS samples further underscores the relevance of our findings and indicates that analysis of macrophages in DCIS lesions might be useful to better identify patients at high risk of carrying disseminated disease.
A84  Proteolytic regulation of the EphB4 receptor in Prostate Cancer; Does it produce bioactive cleavage fragments?

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Introduction
EphB4, a receptor tyrosine kinase, is over-expressed in 66% of prostate cancers (PCa) where it promotes tumour angiogenesis, increases cancer cell survival and facilitates cell invasion and migration; however, the mechanism of action is still unclear. Recently, we developed an overexpression model to study EphB4 in PCa using the cell line 22Rv1 (22Rv1-B4) and identified the presence of novel EphB4 fragments. Using EphB4-specific antibodies directed to either the C- or N-terminus of the protein we predicted these were the result of sequential specific proteolytic cleavage events releasing both extracellular (ECF - 70kDa) and intracellular (ICF - 47kDa) fragments. This study focused on the possible mediators of fragment generation and the function of the ICF in PCa.

Methods and Results
It was determined that the PCa-associated serine protease KLK4 was the mediator of the first cleavage event using recombinant KLK4 on the 22Rv1-B4 cells. The primary cleavage site was determined by N-terminal sequencing to be in the extracellular domain (Arg508), consistent with the identified fragments (70 and 50 kDa). The second fragment of 47 kDa was lost upon γ-secretase inhibition suggesting that the production of the 47 kDa ICF was due to the action of this protease. Subcellular fractionation demonstrated that the 47 kDa fragment was present in the nuclear fraction suggesting nuclear translocation of this fragment. DU145 and PC3 cells engineered to over-express the ICF had a more mesenchymal-like morphology compared to the vector only control cells, suggesting an EMT response, which was further validated by qRT-PCR with EMT markers.

Conclusion
This study provides the first evidence of proteolytic regulation of EphB4 in PCa whereby the PCa-associated protease KLK4 cleaves the ectodomain of EphB4 leading to a subsequent second intracellular cleavage γ-secretase, releasing a potentially bioactive nuclear fragment. We predict this may be a novel mechanism by which EphB4 contributes to the development of PCa. Understanding the function of EphB4 fragments and the proteases that regulate it may identify novel avenues for anti-prostate cancer therapies.
A85 MiR-21 induces myofibroblast differentiation and promotes the malignant progression of breast phyllodes tumors

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Phyllodes tumors (PTs) of breast, even histologically diagnosed as benign, can recur locally and have metastatic potential. Histological markers only have limited value in predicting the clinical behavior of PTs. It remains unknown what drives the malignant progression of PTs. We found that the expression of myofibroblast markers, α-SMA, FAP and SDF-1, is progressively increased in the malignant progression of PTs. Microarray showed that miR-21 was one of the most significantly upregulated microRNAs in malignant PTs compared with benign PTs. In addition, increased miR-21 expression was primarily localized to α-SMA-positive myofibroblasts. More importantly, α-SMA and miR-21 are independent predictors of recurrence and metastasis, with their predictive value of recurrence better than histological grading. Furthermore, miR-21 mimics promoted, while miR-21 antisense oligos inhibited, the expression of α-SMA, FAP and SDF-1, as well as the proliferation and invasion of primary stromal cells of PTs. The ability of miR-21 to induce myofibroblast differentiation was mediated by its regulation on Smad7 and PTEN, which regulate the migration and proliferation respectively. In breast PT xenografts, miR-21 accelerated tumor growth, induced myofibroblast differentiation and promoted metastasis. This study suggests an important role of myofibroblast differentiation in the malignant progression of PTs that is driven by increased miR-21.
Metastatic Osteosarcoma Cell Adaptation to Proteotoxic Stress in the Lung Microenvironment Involves Upregulation of the Endoplasmic Reticulum Chaperone Glucose-Related Protein 78

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Cure rates for pediatric osteosarcoma patients with pulmonary metastases have remained stagnant for the past 30 years. This clinical problem underscores the need to further understand the basic biology that drives metastatic progression in the lung. It has been previously shown by others that metastatic tumor cells experience nitrosative stress within the lung. Nitrosative stress interferes with protein-folding enzymes and can result in the accumulation of unfolded proteins (proteotoxic stress), which if not resolved, can lead to apoptosis. The endoplasmic reticulum (ER) is known to be a key organelle in both sensing proteotoxic stress, and mediating homeostatic responses such as the adaptive unfolded protein response (UPR). We hypothesize that the adaptive UPR is important for metastatic cell survival and growth within the lung. In order to study how metastatic cells adapt to proteotoxic stress in the lung microenvironment, green fluorescent protein (GFP)-expressing high and low metastatic human osteosarcoma cell lines (MG63.3 and MG63, respectively) were studied in the lung explant organ culture system called the pulmonary metastasis assay (PuMA). To survey and compare transcriptional changes in UPR candidate genes between high and low metastatic tumor cells, RNA sequencing was performed on MG63.3 and MG63 cells isolated from homogenized PuMA lung tissue via fluorescence activated cell sorting. A subset of these UPR genes was shown to be upregulated in MG63.3 cells relative to MG63 cells. Of these upregulated genes, Glucose-Related Protein (GRP)78 is known to be an important effector ER chaperone protein in the adaptive arm of the UPR. In situ GRP78 protein expression in metastatic tumor cells within the lung was confirmed by immunofluorescence staining of PuMA lung tissue sections at day 0 and 14. Furthermore, stereological image analysis reveals that MG63.3 cells exhibit a greater area fraction of intracellular GRP78 staining compared to MG63 cells from day 0 to day 14. To ascertain whether down-regulation of GRP78 in highly metastatic cells can inhibit their ability to colonize lung tissue, the small molecule drug IT-139 (Inteqyn), which down-regulates GRP78 expression, was tested for activity in MG63.3 cells in vitro and in the PuMA system. IT-139 was shown to down-regulate GRP78 expression in MG63.3 cells. Furthermore, in combination with the nitric oxide donor molecule PABA/NO (known to induce ER-stress), IT-139 significantly enhanced proteotoxic stress in MG6.3 cells. Moreover, our PuMA studies indicate that IT-139 treatment of MG63.3 cells growing in lung tissue significantly reduced the size of metastatic lesions. Efforts are currently underway to further characterize the effects of IT-139 as monotherapy or in combination with other standard chemotherapeutics in the PuMA system and in pre-clinical animal models of metastatic osteosarcoma. Collectively, the data above suggests that the adaptive mechanisms that metastatic tumor cells use to manage microenvironmental stressors in the lung may be an attractive therapeutic target in the development of novel anti-metastatic therapeutics.
A87 An engineered tumour microenvironment provides new insights into cancer progression and drug responses

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Cancer-associated proteases promote peritoneal dissemination and chemoresistance in malignant progression. In this study, kallikrein-related peptidases 4, 5, 6, and 7 (KLK4-7)-cotransfected ovarian cancer cells were embedded in a bioengineered three-dimensional (3D) microenvironment that contains RGD motifs for integrin engagement to analyse their spheroid growth and survival after chemotreatment. KLK4-7-cotransfected cells formed larger spheroids and proliferated more than controls in 3D, particularly within RGD-functionalized matrices, which was reduced upon integrin inhibition. In contrast, KLK4-7-expressing cell monolayers proliferated less than controls, emphasising the relevance of the 3D microenvironment and integrin engagement. In a spheroid-based animal model, KLK4-7-overexpression induced tumor growth after 4 weeks and intraperitoneal spread after 8 weeks. Upon paclitaxel administration, KLK4-7-expressing tumors declined in size and showed less metastatic outgrowth. KLK4-7-expressing spheroids showed 53% survival upon paclitaxel treatment, accompanied by enhanced chemoresistance-related factors; their survival was further reduced by combination treatment of paclitaxel with KLK (22%) or MAPK (6%) inhibition. The concomitant presence of KLK4-7 in ovarian cancer cells together with integrin activation drives spheroid formation and proliferation. Combinatorial approaches of paclitaxel and KLK/MAPK inhibition may be more efficient for late-stage disease than chemotherapeutics alone as these inhibitory regimens reduced cancer spheroid growth to a greater extent than paclitaxel alone.
A88  Annexin A2 a potential prognostic marker for serous ovarian cancer promotes ovarian cancer metastasis

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Ovarian cancer metastasis is defined by the spread of ovarian cancer cells from the ovarian surface and their implantation to the peritoneum. Using a proteomic approach, we identified annexin A2 to be regulated by ovarian cancer-peritoneal cell interactions. Annexin A2, a calcium-phospholipid binding protein, has been characterized in many malignancies and plays an important role in tumourigenesis. This study investigated the role of annexin A2 using in vitro and in vivo ovarian cancer models and its potential utility as a novel prognostic marker and therapeutic target.

Annexin A2 levels were assessed in a uniform cohort of stage III serous ovarian cancers (n=91) using immunohistochemistry to determine whether annexin A2 can be used as a prognostic marker. Annexin A2 siRNAs was used to evaluate the effect of annexin A2 suppression on ovarian cancer cell adhesion, motility and invasion. Furthermore, annexin A2 neutralizing antibodies were used to examine the role of annexin A2 in invasion and metastasis in vivo using a chick chorioallantoic membrane (CAM) assay and an intraperitoneal xenograft mouse model.

Kaplan-Meier survival analysis of stage III serous ovarian cancers showed high stromal annexin A2 expression was significantly associated with reduced progression free survival and overall survival. Furthermore, multivariate Cox Regression analysis showed stromal annexin A2 was an independent predictor of overall survival in the patients with residual disease. Depletion of annexin A2 significantly inhibited the motility and invasion of ovarian cancer cells and adhesion to the peritoneal cells in vitro. Annexin A2 neutralizing antibodies also significantly inhibited OV-90 cell motility and invasion in vitro and in the CAM assay. Moreover, the growth of SKOV-3/GFP cells and their peritoneal dissemination in nude mice was significantly inhibited by annexin A2 neutralizing antibodies. Our findings suggest that the reduced in tumour burden and metastatic spread is a result of reduced cell survival.

Annexin A2 plays an important role in ovarian cancer metastasis and is therefore a potential prognostic marker and therapeutic target against ovarian cancer.
A89  RNA/DNA differences in Cancer

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RNA-DNA differences (RDDs) result from RNA transcripts that differ from their corresponding genomic sequence by a single nucleotide. This change can theoretically result in functional differences to the protein product. Although RDDs were initially described in normal B-cells, an extensive study of the frequency, location and potential functional impact of RDDs has yet to be described in cancer. We hypothesized that RDDs are generated in cancer cells and may lead to alteration in cancer-associated phenotypes on the single cell level, independent of genomic signature. To test this hypothesis we performed RNA and DNA sequencing (Illumina HiSeq 2000) from six human pancreatic cancer cell lines (MiaPaca2, PanC1, BxPC3, Hs766T, Capan1 and Capan2), pancreas tumors and matching normal tissues from two patients and identified RDDs using a previously established bioinformatics pipeline (Wang et al., Cell Reports 2014). We discovered more than 15,000 and 13,000 candidate RDDs PDAC cell lines and patient tumors. All 12 possible substitutions were observed. These differences were nonrandom as 328 sites were found in all six cell lines, 4464 were common among patient tumor samples and MiaPaca2 cell line. Many of these RDDs are present in coding regions, regulatory elements and stop codons of genes known to be critical in pancreatic cancer biology. For example we identified RDDs in genes involved in epithelial-to-mesenchymal transition and invasion such as PPWD1, CDH1 (E-cadherin), EPCAM, SNAI1, VIM, ZEB1, PRRX1. Thus, RDDs are widespread in cancer cells and many are predicted to affect the protein product of genes relevant to key cancer-related processes, such as invasion and EMT; the generation of RDDs therefore may represent a novel mechanism by which cancer cells can generate vast intercellular phenotypic heterogeneity independent of genomic signature.
A90  Neutrophils promote breast cancer brain metastases

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Brain metastases occur in 20-35% of cancer patients and are associated with poor prognosis due to the lack of efficient therapies. Myeloid cells have been implicated in cancer progression and metastases, and therefore targeting of these cells may have a therapeutic value. Myeloid cells are highly heterogeneous and their phenotype strongly depends on the tissue microenvironment. Our study therefore focused on the unknown role of myeloid cells in brain metastases.

We first confirmed that the presence of primary tumour strongly increased the amount of myeloid cells in the spleen and blood of the mice, as previously reported. The specific role of these myeloid cells in brain metastases was analyzed by antibody-mediated depletion of CD11b+ myeloid cells prior and during the induction of brain metastases, which was performed by administration of cancer cells into carotid artery. Myeloid cell depletion resulted in a significantly reduced growth rate of metastatic lesions in the brain, demonstrating that myeloid cells induced by the primary tumour promote brain metastases. Moreover, depletion of neutrophils with anti-Ly6G depleting antibody also significantly reduced metastatic growth in the brain, thus implicating granulocytic myeloid cells as the critical myeloid cell type involved in brain metastasis. Interestingly, neutrophils infiltrated only leptomeningeal lesions, while parenchymal lesions were devote of granulocytes. In line with their protumoural function, the metastases-infiltrating neutrophils were polarized towards N2 phenotype.
A91 Identification of novel targets in breast cancer brain metastasis

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Between 10-16% of all breast cancer patients eventually develop brain metastasis. Recently, advances in targeted therapy for breast cancer (e.g., trastuzumab, T-DM1, and lapatinib) have prolonged patient survival through better control of the systemic disease; however, the increasing number of patients recurring with brain metastasis represents an emerging challenge. Current treatment options are limited and merely palliative for those patients with brain-metastatic breast cancer, as their median survival continues to be less than one year. To tackle this growing clinical dilemma, we initiated a new direction of investigation to better understand the biological mechanism of brain metastasis and to identify novel targets for intervention, aiming to prolong the survival and improve the quality of life for breast cancer patients diagnosed with brain metastasis.

Kinases activate downstream effectors through phosphorylation of target molecules, placing them at central nodes of cell signaling pathways. In the last decade, the clinical efficacy of kinase inhibitors in cancer therapy has ushered in the era of personalized medicine. However, all existing inhibitors have been developed based on knowledge of primary tumors; no efforts have yet been reported to identify metastasis-specific, druggable molecular targets. As compatible interactions between potentially metastatic tumor cells and secondary organ microenvironments are necessary to result in deadly metastases, we reasoned that kinases nonessential for primary tumor growth may be critical for colonization of the brain and growth of metastases, thereby imbuing metastatic cancer cells with brain-specific growth advantages and serving as ideal therapeutic targets. Therefore, we have performed an unbiased in vivo kinome screen to uncover novel targets for effective brain metastasis therapy.

To achieve this goal, we are overexpressing a kinase library in the MDA-MB-231 breast cancer cell line and performing experimental brain metastasis assays by intracarotid injection into nude mice. The 4 pools assayed thus far have all led to dramatically reduced survival compared to vector control cells, with some even halving the survival time. Next-generation sequencing (NGS) revealed that these aggressive brain metastases were indeed enriched with certain kinases in vivo. Among these were three kinases, SPHK1, FRK, and MAPK12, previously unreported in brain metastasis but with inhibitors available and either FDA-approved or in clinical trials for other pathologies. We are completing screening and NGS of the remaining pools and functionally validating the top candidates in breast cancer brain metastasis. We will then investigate how the candidate kinases confer brain-specific advantages upon brain metastases. Finally, we will explore the potential of using the top candidates as therapeutic targets for the treatment and/or prevention of breast cancer brain metastasis. We will use the pre-existing compounds targeting the kinase(s) of interest to test their ability to treat and/or prevent breast cancer brain metastasis. Targets identified and validated in this manner may serve as the first generation of breast cancer brain metastasis-targeted therapy and help those patients in dire need.
Monday 30th June

B1 STRIPAK components determine mode of cancer cell migration and metastasis

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Cell migration is fundamental to both the development and pathology of multicellular organisms. Migrating cells are strictly dependent on a contractile actomyosin cytoskeleton to generate traction force. The traction force is generated by binding the extracellular matrix (ECM) through integrins, or by binding the plasma membrane through Ezrin/Radixin/Moesin (ERM) proteins where it enables generation of hydrostatic pressure to push the cytoplasm through gaps in the ECM. The molecular ‘tuning’ mechanism that determines whether the actin network is predominantly coupled to the plasma membrane or integrins is not well understood.

We sought to exploit the parallels between border cell migration in Drosophila melanogaster and cancer cell invasion to identify new regulatory mechanisms of cancer invasion and metastasis. RNAi-based screening identified several hundred genes that altered the morphology of the Drosophila egg chamber. Secondary screens of the human homologues in cancer cell lines identified 32 evolutionarily conserved regulators of cell shape. These included three STRIPAK complex components, FAM40A, FAM40B and STRN3. Subsequent analysis revealed that FAM40A negatively regulates MST3&4. These kinases directly phosphorylate ERM proteins and the inhibitors of PPP1CB, PPP1R14A-D, leading to local contraction at the cell cortex. Importantly, translocation of MST3&4 to the cell cortex is mediated by another STRIPAK component, CCM3/PDCD10. Thus MST3&4 locally coordinate the co-localisation of the contractile actomyosin machinery with the ERM proteins in the plasma membrane. Computational modelling predicts that co-localisation of contractile activity and actin-plasma membrane linkage will reduce cell speed on planar surfaces, but favour migration in confined environments similar to those observed in vivo. The predictions are validated by in vitro cell migration assays and in vivo breast cancer metastasis assays.

Finally, we verified the clinical significance of these finding in disease: MST3&4 and CCM3 expression is elevated in the more aggressive sub-type of human breast cancer, while expression of their negative regulators STRN3 and STRN4 decrease. These changes correlate with clinical outcome even within the most aggressive ‘triple-negative’ sub-type of breast cancer.
B2   Genome Scale Prediction of Distant Metastases Sites

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Different cancer types are known to have preferential sites of metastasis. However, the biological mechanisms governing this preference are poorly understood. An arising open research question given the observation that several cancer types metastasize to a specific organ is whether this is a result of genomic features common to all these primary cancers or is it a common functional outcome of different, cancer-specific genomic mechanisms. To address this fundamental question, we developed a novel computational approach that aims to find such a minimal common set, if it exists. Using machine learning techniques, we analyze 165 gene expression profiles of the six different primary tumors (including breast, bladder, liver, endometrial cancer, head & neck and sarcoma) aiming to build a predictor of lung metastases. Employing our novel supervised classification method we obtain prediction accuracy greater than > 85\% on this task, using a standard leave-one-out cross validation procedure. Our analysis identifies a minimal core set of \textasciitilde20 genes that enables high prediction accuracy (> 85\%). This set plays a key role in establishing our predictions and thus potentially involved in determining metastatic site preference to the lungs, in all primary cancer types studied. Larger sets of genes yield relatively similar prediction quality. Analyzing the latter (in particular a set of 200 top ranked discriminatory genes) we find significant enrichment in various metabolic processes. Our analysis hence implies that, at least in the case of lung metastases studied here, a minimal core set of commonly share genes suffices to predict lung cancer metastases as well as any larger set (which includes genes that are primary cancer type specific). This supports the notion that, indeed, there may be common mechanism governing the metastases from different primary tumors to the lung. It suggests new insights into the underlying features of metastasis organotropism to the lungs and predicts novel genetic targets for cancer metastasis prevention.
αB-Crystallin Induction by Matrix Detachment Inhibits Anoikis and Promotes Circulating Tumor Cell Survival and Lung Metastasis \textit{in vivo}

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The ability of metastatic carcinoma cells to survive detachment from the extracellular matrix and escape “anoikis” enables them to disseminate as viable circulating tumor cells and seed distant organs. Although targeting anoikis represents an attractive therapeutic strategy, such efforts have been hampered by our limited understanding of the molecular regulation of anoikis-resistance in cancer. We have identified a novel prosurvival pathway activated by matrix detachment, namely, induction of the antiapoptotic chaperone αB-crystallin previously implicated in the pathogenesis of poor-prognosis solid tumors, including triple-negative breast cancer. αB-crystallin is an antiapoptotic molecular chaperone that has been implicated in the pathogenesis of diverse solid tumors. We examined the potential role of αB-crystallin in regulating anoikis-resistance and metastasis in preclinical models.

The effects of matrix detachment on αB-crystallin mRNA and protein levels in human breast epithelial cells and metastatic carcinoma cells were investigated, and the underlying mechanisms were examined. The effects of stably silencing αB-crystallin on matrix detachment-induced caspase activation and apoptosis in two different metastatic carcinoma cell lines were examined. Additionally, the impact of stably silencing αB-crystallin silencing on circulating tumor cell survival and lung metastasis was investigated using two orthotopic models.

Matrix detachment downregulated extracellular-signal regulated kinase (ERK) activity and increased αB-crystallin protein and mRNA levels. ERK inhibition in adherent cancer cells mimicked matrix detachment by increasing αB-crystallin protein and mRNA levels, while constitutive ERK activation by oncogenic Ras suppressed αB-crystallin induction during matrix detachment. Silencing αB-crystallin in metastatic carcinoma cells increased matrix detachment-induced caspase activation and apoptosis but did not affect cell viability of adherent cancer cells. In addition, silencing αB-crystallin in metastatic carcinoma cells inhibited circulating tumor cell survival and lung metastasis in two orthotopic models, but had little or no effect on primary tumor growth.

αB-crystallin is a novel regulator of anoikis-resistance that promotes lung metastasis at least in part by enhancing circulating tumor cell survival.
B4 A New Lung-Derived Factor Inhibits Neuroblastoma Lung Metastasis by Inducing Cancer Cell Dormancy

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Patients with cancer can develop metastatic disease after latency periods that range from years to decades. This latency can be explained by the occurrence of dormant micrometastatic tumor cells. The mechanisms that trigger dormant tumor cells to “be activated” and subsequently progress to an actively growing metastatic lesion and cause a late relapse are essentially unknown. However, recent data indicate that these mechanisms are largely influenced by the microenvironment of the secondary site. The main goal of our study was to evaluate the role of the lung microenvironment in the regulation of dormancy of neuroblastoma micrometastasis (MicroNB) and their progression toward macrometastasis (MacroNB). We hypothesized that factors present in the lung microenvironment inhibit the propagation of MicroNB cells, preventing them from forming overt lung metastasis. This study indeed shows that lung-derived factors significantly reduce the viability of MicroNB cells, induce a G0-G1 cell cycle arrest accompanied by a down-regulation of Cyclin D1 and decrease ERK and FAK phosphorylation in these cells. Utilizing biochemical separation methods, we isolated and identified the lung-derived factor responsible for the viability inhibitory effect to be mouse hemoglobin subunit beta-2 (HBB2). Purified human hemoglobin beta subunit (HBB) of 147 amino acid residues, which shares a 80% sequence identity to mouse HBB2, had comparable cytostatic/cytotoxic effects on neuroblastoma cells, while the alpha subunit of human hemoglobin was inactive. We identified the inhibitory active fragment of HBB to be a 15 amino acid sequence in the C-terminus of the protein. Treatment with this short inhibitory peptide significantly inhibits neuroblastoma local tumor growth and lung metastasis. HBB2 expression is significantly elevated in the serum and organs of mice harboring neuroblastoma micrometastasis, indicating its potential to serve as a biomarker predicting tumor progression in addition to its potential to serve as a candidate for a new anticancer drug.
Regulation of epithelial mesenchymal transition (EMT) by \textit{Serum Amyloid A 1 (SAA1)} is dependent on integrin in esophageal squamous cell carcinoma (ESCC)

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Esophageal Squamous Cell Carcinoma (ESCC) is the predominant type comprising more than 90% of esophageal cancer, which is a highly metastatic and fatal cancer, and is ranked the eighth in mortality rate in Hong Kong cancer patients (Hong Kong Cancer Registry, Hospital Authority, 2010). Using a functional complementation approach, \textit{SAA1} was identified as one of the tumor suppressor gene candidates. The \textit{SAA1} is an acute phase protein, which is highly expressed in response to inflammation by the liver. It is also present as a secretary protein in histologically normal human epithelial tissues. The expression of \textit{SAA1} was found to be down-regulated in ESCC. Interestingly, the gene expression of \textit{SAA1} and the mesenchymal marker N-cadherin was found to be inversely correlated in a panel of ESCC and the immortalized esophageal epithelial cell lines. Therefore, we want to determine whether \textit{SAA1} could regulate EMT in ESCC.

The mesenchymal markers such as N-cadherin and fibronectin were significantly up-regulated when using \textit{SAA1} knockdown assays by both lentiCRISPR and pLKO shRNA systems in the immortalized esophageal cell lines. At the same time, the expression of another group of cell adhesion molecules, integrin alphaV was found to be concomitantly up-regulated. To elucidate the role of integrin alphaV in regulating EMT-associated genes in ESCC, the knockdown assay of integrin alphaV in ESCC cell line was performed and showed that the expression of mesenchymal markers N-cadherin, fibronectin, and vimentin and EMT-activating transcription factors Twist and Slug, were down-regulated. As a summary, the down-regulation of \textit{SAA1} in ESCC could lead to the up-regulation of integrin and its subsequent induction of EMT. Further investigation will be taken to understand how the regulation of EMT \textit{SAA1} would affect metastasis in ESCC.
B6 Mesenchymal markers in primary tumors and dissemination via hematogenous and lymphatic route in breast cancer patients

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Introduction. Occurrence of epithelial-mesenchymal transition (EMT) in primary breast tumors (PT) is being linked with increased aggressiveness of the cancer. Comparing routes of cancer dissemination and phenotype of disseminated cells in the context of EMT will help to understand metastasis process. The aim of this study was to analyze the expression of EMT markers in PT and correlate them with regional lymph nodes metastases (LNM) phenotype as well as presence of circulating tumor cells (CTCs) markers (epithelial and mesenchymal) in matched pre-operative blood samples of breast cancer patients.

Materials and methods. Primary tumors (N=108), lymph node metastases (N=55) and CTCs-enriched blood samples (N=98) from 108 breast cancer (stage I-III) were investigated. Expression of EMT markers: E-cadherin, N-cadherin, vimentin and SNAIL was analyzed by immunohistochemistry in PT and LNM. Blood samples were drawn before tumor excision and therapy initiation and were subjected to CTCs epithelial marker independent enrichment - negative selection with anti-CD45-covered magnetic particles. Expression of CK19, MGB1, HER2, VIM in CTCs-enriched blood fractions was measured by qRT-PCR. Results of molecular analyses were also correlated with clinico-pathological data of the patients.

Results. Decreased E-cadherin levels (in non-lobular breast cancer) in PT correlated with the presence of CTCs markers (both epithelial and mesenchymal, P=0.05) and lymph node involvement (P=0.019). Overall, tumors with decreased E-cadherin levels showed lymphatic and/or hematogenous spread in 83% of the cases in comparison to 56% of E-cadherin-positive PT. Interestingly, E-cadherin loss was related to increased detection rate of CTC-enriched blood fraction with mesenchymal phenotype (VIM+/CK19-/MGB1+/HER2+) but not with epithelial phenotype (CK19+/VIM-/MGB1+/HER2+) (P=0.005). Mesenchymal CTC-enriched blood fractions were found in 30% of patients with E-cadherin loss in PT and in 5% of PT with normal E-cadherin levels. E-cadherin loss correlated with VIM and SNAIL expression (P=0.06 and P=0.08), but neither VIM nor SNAIL predicted for CTCs presence or their phenotype. Expression levels of analyzed proteins between PT and LNM did not correlate.

Conclusions
Loss of E-cadherin was related to increased hematogenous seeding and lymph node metastases forming potential. Moreover, mesenchymal phenotype of CTC-enriched blood fractions was more frequently observed in tumors with E-cadherin loss.
**B7**  

**[10]-gingerol is a potent inhibitor of breast cancer metastasis in vivo**

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Metastasis is the major cause of death in cancer patients and more effective therapies are required. Epidemiologic studies have shown that Asian populations have lower incidence of breast cancer comparing to western population. This has been attributed in part to differences in dietary habits. In particular, gingerols, natural compound extracted from ginger rhizomes, have been shown to have anti-cancer properties against various tumor cell types in vitro.

We reported recently that [10]-gingerol exerts potent anti-proliferative activity against breast tumor cells in vitro but its efficacy in vivo has yet to be tested. The aim of our study was to evaluate the anti-metastatic properties of [10]-gingerol in vitro and in a murine model of breast cancer metastasis (4T1Br4).

A sulforhodamine B colorimetric assay was used to evaluate the inhibitory effect of this natural product on cell proliferation. In this assay, [10]-gingerol inhibited the proliferation of 4T1Br4 cells with an IC₅₀ of 30µM. In a standard adhesion assay, [10]-gingerol inhibited cell adhesion to laminin-511 and to collagen type IV (50-500µM). [10]-gingerol inhibited colony formation and enhanced the radiosensitivity of tumor cells in vitro. [10]-gingerol was well tolerated in vivo and partially reduced tumour growth. Importantly, [10]-gingerol significantly inhibited spontaneous metastasis to lung and bone.

Taken together, our data indicate that [10]-gingerol is non-toxic and a potent and selective inhibitory drug against metastatic breast cancer.

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B8 Dormancy in a Dish? Growth of Metastatic Breast Cancer Cells in a Bone-Like Microenvironment in Vitro


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Breast cancer metastases may remain dormant in the bone marrow for many years following the initial treatment of the primary tumor. We have developed a 3D model of osteoblasts and matrix which mimics major aspects of the bone environment. In this system, invasive metastatic human MDA-MB-231 breast cancer cells attach to the matrix, form files of single cells, develop invadopodia, infiltrate and partially degrade the collagen matrix. In contrast, under the same culture conditions, the metastasis-suppressed variant, MDA-MB-231BRMS1 cells, display a dormant phenotype. They loosely attach and proliferate very slowly. These same patterns of growth are observed in the femurs of mice inoculated with these cells. We hypothesized that changes in the cytokines or the matrix of the bone-like microenvironment could control the growth or dormancy of the cancer cells. Based partially on anecdotal evidence that bone trauma or breakage is associated with latent metastasis, and on our previous findings that osteoblasts produce inflammatory cytokines in the presence of cancer cells, we added either a cocktail of inflammatory cytokines (IL-6, IL-8, MCP-1, VEGF, GROα) or bone remodeling cytokines (IL-6, TNFα, IL-1β, PGE-2) to cocultures of MDA-MB-231 or MDA-MB-231BRMS1 cells. The inflammatory cytokines had little effect on the morphology or proliferation of either cell type. In contrast, bone remodeling cytokines stimulated the BRMS1 cells to grow vigorously. Of the remodeling cytokines, TNFα and/or IL-1β were sufficient to initiate growth of the BRMS1. We were able to block the response to these cytokines with indomethacin, a cyclooxygenase inhibitor, or with AH6809, an inhibitor to the receptor for PGE2. These data suggest that the growth stimulation of the cytokines was due to prostaglandin production. In another model, MCF-7 cells remained dormant when cultured with FGF on a culture of normal human primary osteoblasts. The addition of bone remodeling cytokines brought about a break in dormancy. However, growth was suppressed when indomethacin or AH6809 was added.

We modulated the matrix by growing the osteoblasts with charcoal-stripped serum to reduced estradiol. The osteoblasts differentiated under these conditions (alkaline phosphatase positive) but did not fully mineralize the matrix (minimal von Kossa staining). In the presence of the modified matrix, both the MDA-MB-231(ER-) and the MDA-MB-231BRMS1(ER-) cells proliferated more vigorously. In conclusion, our data highlight the importance of the matrix and the cells in a bone-like microenvironment in maintaining breast cancer dormancy, and suggest that aspects of the system can be studied in vitro. We are currently determining the mechanisms by which the cytokine microenvironment and matrix alteration can modulate the growth or dormancy of cancer cells in the bone.

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Endothelial C-C chemokine receptor type 5 (CCR5) is required for tumor angiogenesis

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It has recently been proposed that the CCL5/CCR5 axis may be required for endothelial progenitor cell mediated tumor neovascularization1,2. Here, we confirm that while breast tumors grown in CCR5 null mice displayed EPC proliferation defects; tumor growth and angiogenesis were not significantly impaired in wild-type (WT) mice containing CCR5 null bone marrow (BM). This suggested that the tumor vascular defects associated with the CCR5 knockout mouse were primarily due to defects in recruitment of vascular from the surrounding tissue, not the BM. Furthermore, while specific suppression of tumor-CCL5 in vivo, did lead to distinctive BM-associated vascular defects, we show that EPCs express CCR1 implying that CCR5 may be compensated for by expression of other CCL5 receptors in BM mediated vasculogenesis. In support of a paracrine role CCL5/CCR5 signaling in angiogenesis, we show that CCR5 is up-regulated in human and mouse endothelial cells in response to tumor-conditioned medium; and that specific suppression of CCR5 in endothelial cells leads to significant angiogenesis defects. Further, analysis of mouse and human breast tumors led to the identification of a subpopulation of CCR5+ tumor endothelial cells; with a significant correlation between CCR5+ vasculature and adverse clinical course in breast cancer. Taken together, these findings support a role for endothelial cell CCR5 in angiogenesis, tumor growth and spread. Directly targeting CCR5+ vasculature may constitute a novel strategy for inhibiting tumor angiogenesis in breast cancer.

Connexin 43 Deficiency Decreases Tumor Vessel Stability and Augments Angiogenesis and Metastasis

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The association of mural cells (pericytes and smooth muscle cells) with the endothelium of blood vessels regulates blood vessel stability and endothelial quiescence. The decreased mural cell investiture observed on tumor blood vessels allows tumor vessels to be angiogenic. The mechanism(s) by which tumors prevent mural-cell mediated vessel stabilization have not been well elucidated. We have previously demonstrated that heterotypic connexin 43 (Cx43)-dependent gap junction activity is required for mural cell-induced quiescence of endothelial cells, and that exposure of mural cells to media conditioned by aggressive MDA-MB-231 breast tumor cells downregulates Cx43 to release endothelial cells from mural cell-mediated growth inhibition, while media from nontumorigenic MCF10A cells lacks this ability. In this study we examined the impact of host Cx43 deficiency on mammary tumor growth and metastasis. Luciferase-labeled Eo771 mouse mammary carcinoma cells were implanted orthotopically into syngeneic wild type mice or mice heterozygous for Cx43. Primary tumors isolated from Cx43 heterozygous mice showed a 47.7% increase in blood vessel area compared to tumors from wild-type mice as evidenced by CD31 staining. Further, the blood vessels from the Cx43 heterozygous tumors demonstrated a 57.6% decrease in pericyte coverage. The increase in blood vessel density was not associated with increased growth rate or final mass of the primary tumor; however, while no lung metastases were observed in the wild type mice at 14 days after implantation of the primary tumor, 40% of the Cx43 mice demonstrated lung metastases at this time. Together, these data suggest that Cx43 plays an important role in vascular inhibitory and stabilizing interactions, and that its deficiency facilitates tumor angiogenesis and metastasis. Cx43 may therefore serve as a therapeutic target to restore vessel stabilization and inhibit tumor angiogenesis and metastasis. (Supported by NIH CA138727)
The Inhibitor of Differentiation proteins mediate tumour-initiating properties and metastasis in breast cancer.

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There is accumulating evidence that primary breast cancers display phenotypic and functional heterogeneity and that only a minority of cells (tumour propagating cells, TPCs) in a breast tumour are capable of tumour initiation, metastatic dissemination and growth. Identifying factors that regulate the properties of TPCs and how they limit metastatic growth is therefore important for developing strategies to treat patients with metastatic tumours. The Inhibitor of Differentiation 1 (Id1) is a helix-loop-helix protein which functions as a transcriptional regulator. Id1 is highly expressed in metaplastic breast cancers and is required for metastasis in experimental models. However, the mechanism by which Id1 mediates metastasis in breast cancer remains largely unknown.

Our work aims to define the mechanism of Id1 function and investigate whether Id1 and its associated pathways are potential therapeutic targets for the triple-negative breast cancers (TNBC). Examination of ID1 expression profiles in a cohort of breast cancer patients showed that ID1 expression is associated with the TN and HER2-enriched subtypes of breast cancer. Furthermore, ID1 expression is enriched in clinically obtained brain metastases compared to patient matched primary breast cancers. Tumour cells endogenously expressing Id1 were isolated from two different syngeneic mouse models of breast cancer- an Id1-GFP knock-in C3-SV40 Large T transgenic mouse model and a syngeneic p53 null mouse mammary gland tumour model. Phenotypic characterisation demonstrated that the Id1 positive cells are enriched for tumour initiating, self-renewal and metastasis forming potential both in vitro and in vivo. Functional knock down studies using inducible RNAi demonstrated that Id1 are required for tumour propagating functions, both in the context of cell proliferation and self-renewal in vitro, as well as primary tumour growth and during metastatic colonization of the lung in animal models. Transcript profiling experiments revealed several novel Id1 target genes and suggested a regulation of Id1 function by Bmi1/Mel18 and TGF-β signaling pathway. Current directions include defining the transcriptional and proteomic (binding partners) landscape of the rare cells, which are critical for tumorigenesis and metastasis in the TNBC subtype.
B12 Autophagy as a survival response in metastatic cancer growth

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By breaking down and recycling long lived proteins and damaged organelles, autophagy plays an important role in cell homeostasis. During autophagy intracellular components are captured by autophagosomes that fuse with lysosomes where the components are degraded. Autophagy is also a cell survival mechanism activated in situations of stress such as starvation, hypoxia and growth factor deprivation. In tumor development and metastasis autophagy seems to be both suppressive and promoting. Elimination of damaged organelles, unfolded proteins and oncogenic protein substrates prevents malignant transformation of cells. In contrast, autophagy may promote survival of already established tumor cells upon stress. The PI3K-AKT-mTOR pathway is crucial in coordination of a number of cellular processes, including negative regulation of autophagy. To investigate the relationship between PI3K signaling and autophagy, we are using a mouse tumor model of five cell lines originating from the same primary tumor and with different metastatic potential. Using these cells, we found the mTOR activity elevated with increasing metastatic potential. The autophagy markers p62 and LC3B-II were increased in metastatic cells compared to the non-metastatic demonstrating higher autophagy in the aggressive cells. A better understanding of autophagy and PI3K signaling in tumor development and metastasis might allow the development of biomarkers that can improve diagnostics and guide future cancer therapy.
Breast cancer is the second leading cause of cancer related deaths in women and more than 90% of these deaths result from metastatic disease rather than from the primary tumor burden. The primary tumor itself is a heterogeneous mass of cells. Thus a better understanding of metastasis and cancer cell dynamics within the primary tumor will aid in developing efficient strategies to increase patient survival. Twist1 and Snail are two epithelial-to-mesenchymal transition (EMT)-inducing transcription factors that are known to increase metastasis through mechanisms that are cell autonomous. Our data demonstrate that these transcription factors can also non-cell autonomously increase in-vitro invasion and migration, as well as alter the expression of numerous genes and proteins associated with EMT, when conditioned medium (CM) taken from cells expressing these factors is placed on cells not expressing them. We show that the non-cell autonomous effects of Twist1 and Snail are in part induced via the ability of these factors to up-regulate another known EMT-related transcription factor, Six1. Indeed, we show that loss of Six1 downstream of Twist1 and Snail mitigates their ability to non-cell autonomously influence EMT related characteristics including invasion and migration of non-metastatic non-Twist1 or non-Snail expressing cells in-vitro, indicating that Six1 is a key player downstream of these transcription factors. Overexpression and knockdown of Six1 itself, in multiple mouse and human breast carcinoma cell lines, non-cell autonomously influences invasion, migration, and anoikis resistance, as well as alters the expression of EMT-related genes in the non-metastatic non-Six1 expressing cells in-vitro. We also show that Six1 expression in metastatic cells is sufficient to non-cell autonomously increase the metastatic potential of weakly metastatic, non Six1-expressing cells, when these cells are fluorescently tagged and co-injected into mice. Using biochemical methods we have determined that the factor(s) responsible for bestowing the metastatic phenotype is a heat stable protein. Using mass spectrometry approaches, we are identifying the factor(s) secreted by cells expressing these EMT-inducing transcription factors, and assessing the role of the factor(s) in mediating invasion, migration as well as metastasis of neighboring cells that do not express these EMT-inducing transcription factors. Importantly, only a few cells within the primary tumor undergo EMT and have the potential to metastasize and Twist1, Snail and Six1 have the ability to both induce EMT and increase metastasis. Since these transcription factors have also been shown to be associated with poor prognosis and increased metastasis in breast cancer as well as in other cancers, it is critical that we understand not only how they mediate aggressive phenotypes in cells expressing these factors, but also how they mediate non-cell autonomous effects that contribute to increased metastasis of the neighboring non-transcription factor-expressing cells within the heterogeneous tumor.
Metastasis, the foremost cause of cancer mortality, represent the end products of a multistep cell-biological process termed the invasion metastasis cascade. Current evidence overwhelmingly suggests that epithelial-mesenchymal transition (EMT) is one of the early manifestations of metastasis. For this reason, defining the molecular mechanisms that induce and drive EMT have long been the focus of intense investigation. However, the vast majority of these studies have primarily focused on transcriptional regulation associated with EMT. Interestingly though, control over translation through RNA binding proteins and microRNAs dictates which transcripts are translated under different contexts, often independent of transcript abundance, as evident by moderate correlation between transcript abundance and corresponding protein expression. To this end, we have employed polyribosomal profiling to identify translational regulation events in a MCF10A cell line-based model of TGF-β–induced EMT. Our analysis revealed 137 translationally controlled putative positive and negative regulators within this process. Web-based motif prediction algorithms revealed selective enrichment of GU rich elements (GREs) in the 3' untranslated regions (UTRs) of 14 genes whose translation increased in the mesenchymal state, and the majority of these GREs were sufficient to confer translational regulation in \textit{in vitro} reporter assays. We have subsequently identified that CUGBP, Elav-like family member 1 (CELF1) binds the GREs within these transcripts and is robustly induced concomitant with EMT. Induction of CELF1 was both necessary and sufficient to induce EMT \textit{in vitro}, and was due to increased stability of the protein in the mesenchymal cells via suppression of active proteasomal-mediated degradation observed in the epithelial cells. We subsequently utilized RNA-immunoprecipitation coupled with quantitative reverse transcriptase-polymerase chain reaction (RIP-qRT-PCR) and gain and loss-of-function studies to validate and define, respectively, the downstream effectors of CELF1 that are necessary and sufficient to induce EMT. Our current experiments are focused on defining the underlying mechanism that dictates the altered stability of this protein post-TGF-β treatment and the consequent effect on different facets of EMT, both in a cellular model and in the context of model tumors in laboratory mice. Cumulatively our results have defined that regulation of protein expression via CELF1 plays a novel and fundamental role in EMT equivalent in importance to that dictated by the transcriptional response. We anticipate that a more complete understanding of post-transcriptional gene regulation during EMT will help to identify effective new targets for cancer prevention, diagnosis, prognosis, and therapy in breast cancer, and by extension to other solid tumors.
Metastases are responsible for 90% of all cancer deaths, and patients diagnosed with brain metastasis have a dismal 20% probability of one-year survival. Further, breast cancer metastasizes to the brain in approximately 40% of patients who have a tumor that is Her2+. The “seed and soil” hypothesis that frames current investigation of metastasis is uniquely exemplified by the colonization of the brain by circulating breast cancer cells. Accordingly, therapy for brain metastases should not only employ cytotoxic approaches against the tumor cell, but also perturbation of the microenvironment that facilitates cancer cell growth and resilience.

Despite disseminating to distant organs as malignant scouts, most tumor cells fail to remain viable after their arrival. Our previous research shows that the physiologic microenvironment of the brain must become a tumor-favorable microenvironment for successful metastatic colonization by breast cancer cells. These bidirectional interplays of metastatic breast cancer cells and native brain cells are poorly understood and rarely studied. Astrocytes, the prominent glial cells in the central nervous system, are responsible for homeostasis of the brain microenvironment. Furthermore, astrocytes secrete neurotrophins, such as the brain-derived neurotrophic factor (BDNF), which are multifunctional growth factors that play a crucial role in synaptic plasticity, survival, and cognitive function. BDNF binds to the extracellular domain of the Tropomyosin-Related Kinase B (TrkB) receptor. Primary breast cancer patients with TrkB expression present a poor prognosis through cell-resistance to chemotherapy. Therefore, we hypothesize that Her2+ breast cancers express TrkB receptors to exploit BDNF from the brain microenvironment for metastatic colonization. Our results show high expression of TrkB receptor in patient specimens of matched fresh tissue and cells from neurosurgical resections of Her2+ brain metastases (BBMs). Because BDNF expression is highest in the brain microenvironment, paracrine signaling may potentiate tumor cell growth and survival. Indeed, our results show both exogenous and astrocyte-derived BDNF confers a TrkB-dependent cellular proliferation in Her2+ BBMs. In vivo xenograft studies show there is a delay in brain colonization and metastatic growth with BBM TrkB-knockdowns (BBM-KD).

Because Her2 is an orphan receptor which can heterodimerize with ligand-bound receptors, we looked at the possible interaction between TrkB and Her2 in BBM cells. Our results show a strong colocalization of Her2 and phosphorylated TrkB receptor in BBM tissue and cells. Co-immunoprecipitations and 3-D structural modeling confirmed direct binding of Her2 and TrkB receptors. Further, BDNF treatment induces TrkB-Her2 dimerization, which is abrogated in BBM-KD. These results suggest that in order to establish metastatic niche, tumor cells exploit BDNF from the brain microenvironment, resulting in subsequent heterodimerization of TrkB and Her2 receptors, thus promoting proliferative advantage and colonization.
And yet it moves: targeting cytoskeleton dynamics in malignant melanoma

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Malignant melanoma is one of the most aggressive human cancers and its poor prognosis is intimately linked to the spreading of the disease to vital organs such as liver, lung, bones and brain. Although it has been recently shown that drugs targeting BRAF and MEK are effective in melanoma patients whose tumors carry BRAF mutations (Flaerthy et al. 2010, Chapman et al. 2011, Falchook et al. 2012), these drugs lose their beneficial efficacy after a few months of disease control, mostly because of up-regulation of alternative cellular signaling or acquisition of gene amplification and mutations. This problem calls the attention to the need to find more effective drugs for both first line combination as well as second line treatments for relapsed patients. Aiming to discovery new combinatorial approaches that could improve the response of patients to current treatments, we performed an \textit{in vivo}, context-specific screen of potential driver kinases into non-tumorigenic human TERT-immortalized melanocytes (HMEL) bearing oncogenic BRAF (BRAF\textsubscript{V600E}, 89\% of reported BRAF mutations) and inactivation of p53 and RB. Among the 136 wild-type or somatic mutant kinases tested in the primary screen, 9 hits were confirmed in a secondary screen were each of them was singly overexpressed. Here, we will present our most recent data about the validation and the molecular characterization of one of these new therapeutic candidates and we will show how targeting the cytoskeleton dynamics impacts the migration and, ultimately, the metastatic potential of melanoma cells.
Lung adenocarcinoma (LUAD) is a deadly and heterogeneous subtype of non-small cell lung cancer. During LUAD progression, the emergence of alternate epithelial lineage fates in primary tumors correlates with poor outcome. The underlying mechanisms and biological consequences of these phenomena are poorly understood.

Using an integrated approach, we examined the molecular relationship between cell differentiation states, lung cancer subtypes, and clinical outcome, to discover a role for lineage-restricted genes in the pathogenesis of LUAD (1). Specifically, we identified two airway selective lineage transcription factors, GATA6 and HOPX, as inhibitors of metastatic progression. Down-regulation of their function in a subset of human tumors correlates with aberrant tumor differentiation and relapses. GATA6 and HOPX cooperatively restrict the metastatic potential of LUAD cells, by modulating converging transcriptional programs of airway epithelial differentiation, malignant invasion, and metabolic adaptation.

Our findings demonstrate that perturbation of intrinsic cell lineage pathways is a determinant of metastasis in specific lung cancers. The mechanistic implications of these findings for the origin(s) and treatment of LUAD metastasis will be further discussed.

B18  Investigating the function and regulation of CD133 using functional genomics and computational methods

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The cell surface protein CD133 (also known as prominin-1 or AC133) is the most commonly expressed tumour initiating cell (TIC) marker in several hematologic and solid malignancies. These CD133⁺ cells have greater tumorigenic and metastatic potential in xenograft transplantation assays through their increased stemness, resistance to therapies/apoptosis and motility in comparison to their CD133⁻ counterparts. The link between CD133 expression and cancer progression, including the maintenance of a dedifferentiated cell state, in many cancer types demonstrates the necessity of further investigating the biology of this marker.

To this end, we have mined our pooled shRNA drop-out screen data across a large panel of cancer cell lines (Marcotte et al. 2012) to identify genes that are essential (synthetic lethal) in the context of CD133 overexpression. Further, to look for groups of genes acting in similar pathways and processes (analogous to yeast genetics “guilt-by-association”) we developed and utilized a modified mixed-effects model (MEM) computational methodology to cluster these genes based on co-essentiality patterns across many cancer types. From this analysis pipeline, we uncovered a cluster of genes that is highly enriched for transcription factors (TFs) annotated in the literature as being associated with pluripotency and organogenesis. The top hits include multiple members of the Sox family of transcription factors (Sox1, Sox9 and Sox18) as well as Hes family (Hes2 and Hes4). Interestingly, shRNA mediated knockdown of these TFs reduces levels of AC133 expression on the cell surface. Furthermore, the level of knockdown correlates with a decrease in proliferation in CD133 dependent colon cancer cells. As CD133 often marks TICs/metastasis initiating cells, these TFs may be important regulators of either CD133 itself or other key processes associated with it’s expression in TICs.

To expand upon these findings, we are utilizing a computational approach to systematically map the putative binding sites of these TFs genome wide and identify relevant target genes based on co-expression with CD133 by RNAseq and essentiality scores in CD133 expressing cell lines.

This work will afford us a better understanding of the molecular biology surrounding this interesting marker and potentially to the discovery of novel therapeutic targets for the aggressive and invasive subpopulation of therapy resistant cells in patients with cancers of various types.
B19  Novel stochastic tumor models providing evidence for tenascin-C promoting metastasis


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The extracellular matrix molecule tenascin-C (TNC) is highly expressed in most cancers which correlates with a bad survival prognosis in glioblastoma and breast cancer. TNC appears to promote tumor cell proliferation, angiogenesis, invasion and metastasis (1). A TNC substratum can induce cell rounding by interference with syndecan-4/integrin signaling (2), impairing FAK, paxillin, RhoA and Tropomyosin-1 functions (3,4,5) and stimulating tumor cell proliferation (1). TNC can also trigger cell migration (6) and expression of inflammatory cytokines (7), thus demonstrating the versatile functions of TNC.

To better understand the roles of TNC in cancer three mouse models with stochastic tumorigenesis in immune competent conditions and with defined TNC levels (overexpression, knockout) have been established. TNC promoted the angiogenic switch and lung micrometastasis in the Rip1Tag2 insulinoma model involving downregulation of the Wnt inhibitor Dickkopf-1 and activation of Wnt signaling suggesting that TNC creates a Wnt signaling permissive microenvironment (1, 8). In the MMTV-NeuNT breast cancer model a lack of TNC induced an increased tumor latency and less lung metastasis linked to reduced tumor cell survival. In a carcinogen-driven head and neck tumor model the absence of TNC caused a reduced tumor number and progression. Insights into the underlying molecular mechanisms will be provided. These models are powerful novel tools for basic research and targeting TNC in an immune competent context. Our studies have revealed a crucial role of TNC in early and late events of tumor formation and progression.

References:
Role of CD44 in tumor progression and metastasis

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A member of the CD44 family of transmembrane glycoproteins, namely CD44v6 collaborates with Receptor Tyrosine Kinases (RTKs) such as Met and VEGFR-2. In both cases, the co-receptor CD44v6 fulfills two distinct functions. The ectodomain of CD44v6 is required for the presentation of the ligands to the RTKs. Our data indicate that CD44v6 is able to bind both HGF and VEGF in order to promote the activation of their respective receptors, Met and VEGFR-2. In addition, the cytoplasmic domain of CD44v6 in association with ERM (Ezrin-Radixin-Moesin) proteins and the cytoskeleton is necessary for the activation of signaling originating from activated receptors.

CD44v6 also participates in EGF-induced EGFR signalling in breast cancer cells. In contrast, TGFα-induced EGFR signalling is independent of CD44 whereas HB-EGF dependent EGFR activation is dependent on the CD44v3 heparan sulfated isoform. These results suggest a ligand-specific co-receptor function of CD44 for EGFRs.

The study of the co-receptor function of CD44v6 for RTKs allowed the identification of peptides that can block their activation in vitro and in vivo. These peptides have been used in highly metastatic rat and human pancreatic carcinoma models where they drastically inhibit growth of tumors and metastatic spreading of the cancer cells. Most excitingly, the CD44v6 peptides eliminate already established pancreatic cancer metastases suggesting a role of CD44v6 in the survival of metastatic cells in distant organs. Treatment of 4T1 breast tumors with the CD44v6 specific peptide led to a reduction of vascularisation of the primary tumor and to a reduction of metastases in the lung indicating that CD44v6 is also involved in breast cancer.

Altogether, more and more evidence reveal the plasticity of the CD44 family of proteins and point towards a ligand-specific recruitment of the various CD44 isoforms by RTKs. These multiple collaborations between CD44 isoforms and RTKs allow their participation in various types of cancers including pancreatic and breast cancers.
B21 Uncovering a New Role for Peroxidases in Breast Cancer Development and Metastasis

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Micro-environmental changes associated with extracellular matrix (ECM) remodeling including fibrosis and angiogenesis is a hallmark of tumour development and metastasis. Peroxidases are heme-containing enzymes whose functional role in human health has mainly been limited to providing a mechanism for oxidative defence against invading bacteria and other pathogenic microorganisms. Myeloperoxidase (MPO) and eosinophil peroxidase (EPO) have often been associated with fibrotic tissue in various organs and have long been shown to be present at elevated levels in a variety of tumour types including breast cancer. However, their potential contribution to tumourigenesis and metastasis has not been described.

In this study we investigated the potential of peroxidase enzymes to promote tumour growth and metastasis using the 4T-1 orthotopic breast cancer model and dissected the molecular mechanisms by which peroxidases mediate this effect in the context of cell migration, invasion, angiogenesis and ECM biosynthesis.

Our data clearly show peroxidase enzymes possess a novel and well conserved capability to stimulate mammary fibroblasts to secrete collagenous proteins and generate a functional ECM that is dependent on peroxidase catalytic activity. Using human umbilical vascular endothelial cells (HUVECS), we also showed that peroxidases promoted cell migration and invasion while also stimulating angiogenesis in vivo, as assessed by a matrigel plug assay. Additionally, peroxidase treatment of 4T-1 mouse breast cancer cells in vitro, promoted a concentration-dependent increase in migration and invasion of cancer cells with no effect on cell proliferation. In the syngeneic 4T-1 orthotopic mouse breast cancer model, peroxidase treatment increased mammary tumour burden and promoted pulmonary and hepatic metastases. Histological assessment of the primary tumours from peroxidase treated animals demonstrated significant increases in tumour associated collagen deposition and vessel formation.

Taken together, these data are the first to demonstrate that peroxidases released by neutrophils and eosinophils within the tumour stroma possess pro-tumourigenic and pro-metastatic activity and may not just be a line of host defence as previously thought. Understanding the molecular mechanism(s) by which peroxidase proteins drive tumourigenesis will be critical in the development of peroxidase inhibitors as new strategies for breast cancer prevention and therapy.
B22  BIX02189 binds to activin receptor-like kinase 5 and inhibits the pro-metastatic activity of transforming growth factor-β1 in human lung carcinoma A549 cells.

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Transforming growth factor-β (TGF-β) promotes tumor metastasis by inducing an epithelial to mesenchymal transition (EMT) in cancer cells. The MEK5 [MAPK (mitogen-activated protein kinase)/ ERK (extracellular-signal-regulated kinase) kinase 5]/ERK5 pathway has been implicated in cancer progression and development. In this study, we investigated the effects of MEK5 pathway-specific inhibitors, including BIX02189 and XMD8-92, on pro-metastatic responses induced by TGF-β1-induced EMT, cell migration, and expression of matrix metalloproteinase-2. However, the effect of BIX02189 on TGF-β1-induced EMT was not due to the inhibition of MEK5 pathway, as this effect was not observed upon depletion of MEK5 or ERK5 with small interference RNA. Moreover, TGF-β1 had no stimulating effect on the expression of phosphor-ERK5 (Thr218/Tyr220). Instead, BIX02189 significantly suppressed Smad3 C-terminus phosphorylation, nuclear translocation and transcriptional activity in TGF-β1-stimulated A549 cells. Pull-down assay data revealed the binding of BIX02189 to TβRI, and a docking and molecular dynamics simulation confirmed binding of BIX02189 as a TβRI inhibitor that may be useful for preventing the progression of advanced cancer.
**B23  Functional and molecular characterization of cancer stem cell heterogeneity in human squamous cell carcinomas**

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Metastasis is the main cause of cancer related death, yet the tumor cells that generate metastasis *in vivo* have not been identified so far. This is particularly relevant in tumors that display a very high metastatic rate, such as oral squamous cell carcinoma (SCC), the 5th most diagnosed tumor in industrialized countries, or tumors that may not show a high metastatic rate, but that are highly prevalent such as cutaneous SCC (2nd most diagnosed tumor).

Cancer Stem Cells (CSCs) display a great functional and molecular heterogeneity and it has been proposed that different CSCs subclones exist in the tumor: some of them might have the ability to generate metastasis, behaving like metastasis-initiating cells.

We have developed a novel orthotopic model of human cutaneous and oral SCC, which is allowing us to study tumor cell heterogeneity *in vivo*.

Microarray analysis of Label-retaining and proliferative CSCs reveals that slow-cycling cells express a unique signature that might confer them the potential to colonize distant organs, suggesting that exists a CSC population already programmed to metastasize to specific organs.

Further, we have developed a system to track and interrogate functional relationships and tumoral potential between different CSCs populations.

Detailed functional characterization of slow-cycling versus proliferative CSCs will help us to understand the significance of the LRC population in SCC tumoral and metastatic processes.
B24 Molecular profiling of EpCAM-positive cells isolated from the bone marrow of breast cancer patients

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Metastasis is the major cause of cancer-related death. To prevent metastasis it is necessary to identify the molecular characteristics of disseminated cancer cells (DCCs), the putative metastasis founder cells. DCCs are currently detected in the bone marrow (BM) of breast cancer (BC) patients by staining against epithelial markers, such as cytokeratins or the epithelial cell adhesion molecule (EpCAM). The surface marker EpCAM allows the isolation of viable cells and therefore isolation of cellular DNA and mRNA. The aim of this work was to detect and identify putative metastasis founder cells among the EpCAM-positive cells isolated from the BM of BC patients. Bone marrow samples of breast cancer patients were screened to isolate EpCAM-positive cells. Detected EpCAM-positive cells were isolated and their genomic DNA (gDNA) and total mRNA were subjected to whole genome and transcriptome amplification. Subsequently cDNA and gDNA was analysed by sequencing, PCR and gene expression analysis. To get a first glimpse into the biology of DCCs from breast cancer we investigated the expression of EpCAM, estrogen and progesterone receptor (ESR1 and PGR) and asked whether the PIK3CA gene is mutated in DCCs. Finally, we compared DCCs of patients in M0 stage disease and of patients with M1 stage disease.

In 50% of analysed samples we detected single EpCAM-positive cells. In total, we isolated 277 single cells. After whole genome and whole transcriptome amplification 58% of cells had a good quality gDNA and 69% a good quality cDNA. 64% of BM samples were obtained from luminal A/B subtype of BC patients. Surprisingly, only 3% of analysed cells expressed ESR1 transcripts and no cells expressed PGR. Expression of EpCAM was detected in 80% of cells. Only 2% of the cells harboured PIK3CA mutations, a value much lower than that observed in circulating tumour cells from M1 stage patients. This difference between M0 and M1 stage DCCs was also seen for gene expression profiles. Here, cells from metastatic patients overexpressed differentiation and proliferation markers (e.g. RANKL), which were not observed in M0-DCCs. In summary, our findings suggest that the biology of M0-DCCs needs to be investigated in much greater detail to unravel candidate therapy targets and mechanisms of adaptation and progression to life-threatening disease.
B25  Crosstalk of tumor cells and lymphatic endothelium in melanoma metastasis

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The aggressive skin tumor, melanoma, is the most rapidly increasing tumor type in Caucasian people. Although surgical removal of the lesion serves as an effective treatment for early stage melanoma, metastatic melanoma is currently practically incurable and the patients have very poor prognosis. The involvement of the lymphatic system in melanoma progression is well established and also used as a prognostic factor. However, the specific mechanisms and consequences of the melanoma cell and lymphatic endothelial cell (LEC) interactions are poorly understood. To this end, we have developed several cell co-cultivation systems, including co-cultures of the two cell types on different matrix-coated surfaces, as well as novel three-dimensional (3D) cultures. In the 3D co-cultures single melanoma cells are grown together with LEC cell aggregates also called spheroids in a 3D cross-linked fibrin. This has allowed us to study the alterations in the melanoma cell invasion and proliferation as well as changes in the LEC phenotype and function. Our results show gain of invasive properties in the melanoma cells and recapitulate invasion into the LEC spheroids with concomitant downregulation of the endothelial markers in the LECs. We observe intriguing changes in the LEC master regulator Prox-1 expression, as well as alterations in the surface and cell-cell contact markers of the co-cultured LECs. In the co-cultured melanoma cells, we detect increased levels of invasion and metastasis related chemokine receptor, CXCR4. Our preliminary results indicate that a CXCR4 antagonist treatment affects the LEC spheroid invasion. Furthermore, we have investigated tumor formation and metastatic potential of the 3D co-cultured and LEC primed melanoma cells in vivo in mice. The results from the xenograft model of co-cultured melanomas and LECs suggests that preincubation of LECs with melanomas would generate a more pronounced stromal microenvironment in the tumors and increase the metastatic capacity of the tumor cells.
LOX expression is associated with infiltrative growth in Glioblastoma

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Patients diagnosed with glioblastoma exhibit a very poor survival, with a median of 16 months with standard care of resection, radiation and chemotherapy. Relapse and the ensuing morbidity and mortality, is attributed to the recurrent growth of infiltrative glioblastoma cells. To date, there is a poor understanding of the regulation and interaction of cellular pathways underlying the invasive behaviour of these infiltrative glioblastoma cells.

Lysyl oxidase (LOX) is a hypoxia-regulated, extracellular matrix enzyme that catalyses the cross-linking of collagens and elastin. We have previously reported that LOX expression is up regulated and associated with invasion, metastasis and poor patient survival in various solid tumours.

Here, in a publically available glioblastoma patient dataset of 205 patients, we have found a correlation between malignant glioma with a mesenchymal signature and high LOX gene expression; additionally, patients with high LOX expression have significantly poorer survival. Utilising infiltrative orthotopic glioblastoma models, we showed that LOX expression is predominantly expressed in the infiltrating cells, supporting a role for LOX in aggressive glioma progression.

Early analysis of both chemical and genetic inhibition of LOX shows that the infiltrative behaviour of the glioblastoma cells can be modulated both in vitro and in vivo by LOX. We are currently combining gold standard chemotherapy (temozolomide) with the small molecule LOX inhibitor (β-aminopropionitrile) in our in vivo xenograft models. The full extent of LOX manipulation is currently being characterised.

Our work will provide critical insight into infiltrative glioma and the potential role of LOX in the infiltrative phenotype, and allow us to determine whether co-targeting LOX will provide additional therapeutic benefit for patients with glioblastoma.
B27  The identification and functional implications of soluble bone-derived factors on breast cancer cell migration

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Metastatic breast cancer has an affinity for certain organs and tissues, a phenomenon termed organ tropism. Of particular interest is breast cancer’s preference for bone, since this is the most common site of metastasis in breast cancer patients. In addition, research suggests that breast cancer cells that do metastasize may have stem-like properties, including the ability to self-renew and differentiate into a heterogeneous tumor. These cells can be identified by their high aldehyde dehydrogenase activity (ALDH) and/or CD44⁺CD24⁻ phenotype. However, it is unclear whether properties of the organ microenvironment or the stem-like cancer cells (or both) facilitate metastatic organ tropism. In the current study, we tested the hypothesis that bone marrow-conditioned media (an ex vivo representation of the bone microenvironment) contains specific soluble factors that enhance the growth and migration of whole population and ALDHhiCD44⁺CD24⁻ breast cancer cells. Bone marrow-conditioned media (BMCM) generated from the bones of athymic nude mice was analyzed for the presence and identity of soluble factors using protein arrays. Several factors were identified and osteopontin (OPN), matrix metalloproteinase-14 (MMP-14) and insulin-like growth factor-2 (IGF-2) were chosen for further investigation into their role as potential mediators of breast cancer cell migration to bone. Validation of expression by ELISA confirmed that OPN and MMP-14 were present in the BMCM in significant amounts while IGF-2 was not. OPN was depleted from the BMCM using immunoprecipitation and migration of MDA-MB-231 and SUM-159 breast cancer cells to the BMCM was assessed. Results indicate that bone-derived OPN significantly enhances MDA-MB-231 and SUM-159 breast cancer cell migration (P<0.05). Additionally, bone-derived OPN enhances the migration of ALDHhiCD44⁺CD24⁻ MDA-MB-231 breast cancer stem cells relative to their ALDHlowCD44⁺CD24⁻ counterparts (P<0.05). The interaction between bone-derived OPN and its cell surface receptors CD44 and αvβ5 in breast cancer cell migration to BMCM was also investigated using blocking antibodies. We observed that both whole population and ALDHhiCD44⁺CD24⁻ MDA-MB-231 breast cancer cells interact with bone-derived OPN via CD44 and αvβ5 (P<0.05). Ongoing studies are investigating the activation of OPN-mediated signaling pathways in breast cancer cells as well as the role of MMP-14 in breast cancer migration to bone. Overall, elucidation of the interactions between bone-derived soluble factors and breast cancer cells could contribute to future development of novel therapeutics that interrupt these interactions in the bone marrow niche, thereby improving breast cancer patient prognosis.
A mitochondrial switch promotes tumor metastasis

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Cancers evolve a subpopulation of tumor cells that metabolically rely on glycolysis uncoupled from oxidative phosphorylation irrespectively of oxygen availability (aerobic glycolysis). Given that most metastases are abnormally avid for glucose (which is the rationale for their clinical detection using FDG-PET) and because clinical data show a positive correlation between lactate production and tumor metastasis, we reasoned that cells performing aerobic glycolysis could constitute a population of metastatic progenitor cells that would remain glycolytic in the blood stream. We found a different metabolic phenotype, though. Indeed, using serial rounds of in vitro selection of highly invasive tumor cells (starting from wild-type SiHa human cervix adenocarcinoma cells) and in vivo selection of supermetastatic tumor cells (starting from B16-F10 mouse melanoma cells), we identified a mitochondrial switch corresponding to an overload of the TCA cycle with preserved mitochondrial functions (including ATP production) but increased mitochondrial superoxide production. The switch provided a metastatic advantage which was phenocopied by moderate OXPHOS inhibition associated with mild mitochondrial superoxide increase. Both conditions involved protein tyrosine kinase PTK2B/Pyk2 expression and Src activation as downstream effectors. Thus, two different events, OXPHOS overload or moderate OXPHOS inhibition, promote superoxide-dependent tumor cell migration, invasion, clonogenicity, and metastasis; demonstrating the central role of mitochondrial superoxide generation in the pathogenesis of metastasis. Consequently, we report that mitochondria-specific superoxide scavenging (using mitoTEMPO or mitoQ) inhibits metastatic dissemination from primary mouse and human tumors, which opens a new avenue for the therapeutic prevention of tumor metastasis.

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Autocrine Laminin-511 Modulates EMT and Chemoresistance in Triple Negative Breast Cancer and is Required for Bone Metastasis

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The matrix protein laminin (LM)-511 ($\alpha_5\beta_1\gamma_1$ trimer) is abundant in triple negative breast cancers and promotes adhesion and migration through ligation of $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrin receptors. Its expression correlates with disease progression in patients and with bone-metastatic ability in a mouse model of spontaneous breast cancer metastasis. However, direct evidence of its functional contribution to metastasis in vivo is lacking. Here we demonstrate for the first time that stable downregulation of LM-511 ($\alpha_5$ subunit) in aggressive metastatic variants of the 4T1 mammary carcinoma model (4T1BM2) dramatically impairs their ability to metastasise spontaneously to bone but not their growth in the mammary gland.

Moreover, down regulation of the LM-511 receptor, $\alpha_6\beta_4$ integrin (B4 subunit) almost completely inhibited 4T1BM2 experimental metastasis to bone. In vitro, integrin B4 downregulation decreased invasion and colony formation, indicating that LM-511/integrin $\alpha_6\beta_4$ interactions may be required for invasion and survival in bone. We provide evidence that stromal factors in the bone microenvironment activate Akt signaling in tumor cells and cooperate with LM-511 to induce these responses and efficient metastasis.

Suppression of LM-511 expression in 4T1BM2 or human MDA-MB-231 cells was associated with changes reminiscent of a Mesenchymal to Epithelial Transition (MET). Specifically, reduced expression of LM-511 induced a reversion from a motile/mesenchymal phenotype to a more epithelial-like morphology as evidenced by the formation of tightly packed epithelial patches in standard culture. Further analysis in 4T1BM2 cells showed that these changes were associated with classical features of MET including increased E-cadherin and reduced N-cadherin, SNAIL, TWIST, ZEB-1, ZEB-2 and vimentin expression in vitro and in vivo. Attachment to exogenous LM-511 rescued this phenotype and induced re-expression of mesenchymal markers, as seen in control cells. Consistent with the proposed chemoprotective role of EMT and matrix proteins, suppression of LM-511 or treatment with lebein-1, a potent snake-derived disintegrin that targets $\alpha_3\beta_1$ integrin in these cells, inhibited motility and invasion in vitro and enhanced their sensitivity to doxorubicin or Paclitaxel.

Collectively, these observations indicate that targeting LM-511/integrin interactions could provide an effective strategy to inhibit metastasis in chemoresistant triple negative breast cancer.
B30 Bone-Metastatic Triple Negative Breast Tumors Require αvβ3 Integrin for Early Dissemination

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Although many pre-clinical studies have implicated β3 integrin receptors (αvβ3 and αIIbβ3) in cancer progression, β3 inhibitors have shown only modest efficacy in patients with advanced solid tumors. We propose that the limited efficacy of β3 inhibitors in patients could arise from our incomplete understanding of the precise function of β3 integrin and, consequently, inappropriate clinical application. Data from animal studies are conflicting and indicate heterogeneity with respect to the relative contribution of β3-expressing tumor and stromal cell populations in different cancers. Here we aimed to clarify the function and relative contribution to metastasis of tumor versus stromal β3 integrin in clinically relevant triple negative models of spontaneous breast cancer metastasis, with particular emphasis on bone metastasis.

We show that stable down-regulation of tumor β3 integrin dramatically impairs spontaneous (but not experimental) metastasis to bone and lung without affecting primary tumor growth in the mammary gland. Unexpectedly, orthotopic tumor vascularity, growth and spontaneous metastasis are not altered in mice null for β3 integrin. Tumor β3 integrin promotes migration, protease expression and trans-endothelial migration in vitro and increases vascular dissemination in vivo, but is not required for bone colonisation in experimental metastasis assays. We conclude that tumor rather than stromal β3 expression is essential and is required early for efficient spontaneous breast cancer metastasis. Accordingly, high β3 expression shows a strong association with early metastasis and shorter disease-free survival in breast cancer patients with ER-ve tumors. Thus, we propose that β3 inhibitors may be more efficacious if used in a neoadjuvant setting rather than after metastases are established.
B31  Pro-metastatic protein S100A4 stimulates melanoma cell motility and dedifferentiation accompanied by activation of glycolytic metabolism

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S100-family proteins are known to promote metastasis; S100A8/A9 has been associated with metastatic niche, while S100A4 has been linked to enhanced cellular motility. To investigate further how S100A4 as a microenvironment factor can promote acquisition of a motile phenotype, we evaluated the response to recombinant protein S100A4 in metastatic melanoma cells. We used two cell lines, Melmet 1 and Melmet 5 demonstrating motile dedifferentiated and non-motile differentiated phenotype, respectively. While Melmet 1 did not show particular changes in response to S100A4, the migration and invasion of Melmet 5 was potentiated by \textasciitilde2-fold. Further, the expression of melanocytic differentiation genes, such as MITF, the master regulator of the lineage, and its targets was reduced in S100A4-treated Melmet 5, particularly in those cells that migrated. In addition, global gene expression analysis revealed S100A4-dependent modulation of metabolism-associated genes linked to NAD/NADH system (KYNU, NAMPT and NNMT), antioxidant capacity (SOD2 and metallothioneins) and glycolysis (LDHA). To investigate whether S100A4 has any impact on metabolism, a number of metabolic parameters were measured. It was observed that S100A4-treated Melmet 5 cells had reduced mitochondrial activity and increased glucose consumption and lactate production, suggesting a switch towards glycolytic energy production. To characterize further the metabolic alterations in S100A4-responding cells, we are currently using \textsuperscript{13}C NMR to study the metabolic fate of [1,2-\textsuperscript{13}C] glucose.

Altogether, our data indicates that extracellular S100A4 promotes acquisition of dedifferentiated and motile phenotype associated with activated glycolytic metabolism. This observation supports the concept that metastasis promoters like S100A4 stimulate the switch from mitochondrial oxidative metabolism to glycolysis, the Warburg effect, which is beneficial for disseminating cells.
B32  Metastasis is facilitated by selectin-driven monocyte recruitment

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The formation of emboli by association between circulating tumor cells, leukocytes and platelets enables tumor cells to survive in the blood stream and facilitates metastatic efficiency. The cell-cell interactions leading to the embolus formation and arrest of tumor cells in the vasculature are mediated by selectins. However, the role of non-tumor derived selectin ligands during the development of metastasis remains unclear.

In this study we examined Fucosyltransferase-7 (Fuc-TVII\(^{-/-}\)) deficient mice which express minimal amount of functional selectin ligands. The experimental metastasis model revealed that Fuc-TVII\(^{-/-}\) mice have attenuated metastasis upon injection of the carcinoma cells MC-38GFP, 3LL and melanoma cells B16/BL6 when compared to C57/BL6 (wt) mice. Although we observed no difference in initial seeding of tumor cells between mouse models, the apoptosis of tumor cells was elevated in Fuc-TVII\(^{-/-}\) lungs compared to wt mice. This data shows the important function of non-tumor derived selectin ligands in metastatic formation.

To assess which selectin ligands are involved in this process we prepared chimeric mice. Interestingly, Fuc-TVII\(^{+/+}\) chimeric mice expressing selectin ligands only in the hematopoietic compartment rescued metastasis to comparable levels as in wt controls. We detected lower levels of the monocyte chemotactic cytokine CCL2 in the lungs of Fuc-TVII\(^{-/-}\) mice, which led us to further investigate leukocyte recruitment. We observed reduced monocyte numbers in Fuc-TVII\(^{-/-}\) lungs compared to controls. Moreover, we showed that decreased interaction of monocytes with tumor cells is associated with lower metastatic efficiency and tumor cell survival. Adoptive transfer of Fuc-T7+ monocytes rescued metastasis in Fuc-TVII\(^{-/-}\) mice upon injection of MC-38GFP and 3LL cells, proving the essential function of selectin-mediated monocyte recruitment during metastasis. The analysis of putative selectin ligands on monocytes revealed P-selectin glycoprotein ligand-1 to be the major adhesion molecule on leukocytes required for endothelial adhesion. This study indicates that selectin ligands on leukocytes promote monocyte recruitment and enable tumor cell survival, extravasation and metastasis.
B33 The crucial impact of the unique organ-specific defense system during colonization and outgrowth of brain metastasis

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The exposure to external and endogenous threats varies tremendously in frequency and intensity in the organs. While the liver has to constantly deal with pathogens flooded by the portal vein, other tissues are extremely protected from these danger molecules or pathogens, in particular the brain. In addition, the brain has a unique defense system consisting of microglia and astrocytes. To make thinks more complicated, microglia does not even derive from the bone marrow monocytes, they are a unique macrophage-like population which descend from embryonic cells of the yolk sac.

However, until now the impact of these unique organ defense systems are almost unknown during the crucial steps of metastasis, the distant colonization and outgrowth. Recently, we demonstrated that microglia, could also have metastasis-promoting effects. However, our further investigations in vitro and syngeneic mouse models reveal that astrocytes and microglia intend to fight against the invaders. Unfortunately, this attack is not always effective; in contrary sometimes it may even promote the growth of metastasis. Additionally, we could also reveal significant differences of the CSF1 dependency. While the BMDMs are addicted to CSF1, microglia is not. This result further strengthen the difference of this unique macrophage-like population.

Taken together, our data reveal an important if not crucial role of the unique organ defense systems during metastatic colonization and outgrowth, in particular in brain metastasis.
HOTTIP expression is associated with metastasis formation, predicts outcome and is altered in plasma samples of hepatocellular carcinoma patients

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Hepatocellular carcinoma (HCC) is among the leading causes of cancer-related death. Despite the advances in diagnosis and management of HCC, the biology of this tumor remains poorly understood. Recent evidence highlighted long non-coding RNAs (lncRNAs) as crucial determinants of HCC development. Here, we report that the lncRNA HOTTIP is significantly up-regulated in HCC specimens. HOTTIP gene is located in physical contiguity with HOXA13 (a member of the HOXA gene family) and directly controls the HOXA locus genes expression via the interaction with the WDR5/MLL complex. HOXA genes encode transcription factors regulating embryonic development and cell fate. Here, by correlating clinico-pathological and expression data, we demonstrate that HOTTIP and HOXA13 levels are associated with HCC patients’ clinical progression (i.e. metastasis formation) and predict disease outcome. In contrast to the majority of other studies, our data are obtained from snap-frozen needle HCC biopsies matched with their non-neoplastic counterparts from patients that had not received any therapeutic treatments and presented with no metastasis at the time of biopsy collection. In addition, using a panel of 20 liver cancer-derived cell lines, we uncover a novel bidirectional regulatory loop between HOTTIP/HOXA13; demonstrating for the first time that a lncRNA target (HOXA13) can feed back to its regulator (HOTTIP) to influence its expression. In vitro gain and loss of function experiments revealed that HOTTIP can regulate liver cancer-derived cells proliferation. Furthermore, our preliminary data suggest that HOTTIP is also increased in the plasma of HCC patients, making it a suitable biomarker candidate for HCC detection. Conclusion: our study highlights the key role of HOTTIP in HCC development by associating its expression to metastasis and survival, provides novel insights on lncRNA-driven hepatocarcinogenesis and paves the way for further investigation about the possible role of HOTTIP as predictive biomarker of HCC.
B35 RELN as a tumour suppressor in head and neck squamous cell carcinomas

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Head and neck squamous cell carcinoma (HNSCC) causes 300,000 deaths worldwide annually. Increased number of lymph nodes with metastatic lesions and the presence of extranodal spread are strong predictors for distant metastasis and poor survival of the patient. Despite advances in treatment, there has been little improvement in the survival of patients with metastatic HNSCC. Therefore, better understanding of the molecular mechanisms underlying progression and metastasis of HNSCC is important for better prevention and treatment.

Reelin (RELN) is a large secreted extracellular matrix glycoprotein that is expressed in many tissues. In neurons, RELN interacts with two transmembrane receptors, very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2). Binding RELN to these receptors leads to activation of Src Family Kinases (SFKs) and phosphorylation of the intracellular adaptor protein Disabled-1 (DAB1). In macrophages, RELN activation of VLDLR triggers disabled homolog-2 (DAB2) phosphorylation and signaling. In HNSCC, downregulation of DAB2 switches TGF-β from a tumour suppressor to a tumour promoter. Recent genomic sequencing studies have shown that RELN is frequently mutated in HNSCCs and SCCs of skin and lung. However, the role of RELN in tumour pathogenesis of SCCs remains unknown. We hypothesised that RELN plays an important role in keratinocyte homeostasis and disruption of its function contributes to carcinogenesis.

To address this hypothesis, we stably knocked down RELN in SCC-derived human oral keratinocyte cell lines, UM-SCC4, HN13 and SCC15, and analysed their behaviour. RELN knockdown increases the proliferation and migration of SCC cell lines in vitro. Furthermore, treatment with TGF-β induces RELN knockdown cells to dissociate from one another, whereas this effect was less prominent in control cells. Preliminary data shows that exposure of UM-SCC4 to TGF-β increases the proliferation and migration in RELN knockdown cells, but not in control cells. Western blot analysis shows that both DAB1 and DAB2 are expressed in UM-SCC4 cells. However, TGF-β reduces only phosphoDAB2 levels but not phosphoDAB1 (Y232) levels. Moreover, the exposure of the knockdown UM-SCC4 to TGF-β reduces DAB2 levels whereas RELN protects them from this effect, thereby preventing a positive feedback loop of pro-malignant TGF-β signalling from occurring. These results suggest that inactivation of RELN signaling in keratinocytes might contribute to SCC progression by switching their response to TGF-β from a tumour suppressive to a tumour promoting pathway.
Characterization of Cancer Associated Fibroblasts in mammary gland carcinoma and lung metastasis

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While the crucial role of the tumor microenvironment in tumor initiation and progression is well established, the role of the metastatic microenvironment, and in particular, of cancer-associated fibroblasts (CAFs) in facilitating metastatic growth of disseminated tumor cells is largely unknown. Our Lab has uncovered a novel role for cancer-associated fibroblasts (CAFs) in mediating tumor-promoting inflammation in several tumor types, including breast cancer. However, the role of CAFs in supporting the formation of a permissive metastatic niche that enables metastatic growth of disseminated breast cancer cells is unresolved.

CAFs are a heterogenic population composed of several sub-populations with different phenotypes. This heterogeneity is manifested in part, by a partial overlap in the expression of various mesenchymal markers. Whether or not these different phenotypes correlate with distinct functional roles in the promotion of tumor growth is yet to be investigated.

To characterize the various sub-populations of fibroblastic cells during progression of mammary carcinoma and lung metastasis, we performed an immunofluorescent morphometric analysis using several known mesenchymal markers in a transgenic mouse model of breast carcinoma – the MMTV-PyMT model. At the primary tumor site, co-labeling with α-SMA, a known marker for activated fibroblasts, and PDGFRα, previously shown to be a robust marker for normal fibroblasts, revealed an increase in α-SMA expression within the PDGFRα\textsuperscript{+} population, indicating resident fibroblasts that undergo gradual activation are one origin for CAFs. Similarly, we show a gradual increase in α-SMA expression in lung metastases, indicating that fibroblast co-activation is operative at the metastatic site.

To our excitement, we observed a gradual decrease in the proportion of PDGFRα\textsuperscript{+} fibroblasts within the growing α-SMA\textsuperscript{+} population, urging us to look for other sources from which CAFs are recruited. Using adaptive bone-marrow (BM) transplantation of whole BM from PyMT+GFP\textsuperscript{+} and GFP\textsuperscript{+} mice into PYMT and FVB/n mice, respectively, we show that a fraction of CAFs present in mammary tumors are BM-derived. This sub-population is PDGFRα\textsuperscript{−}, suggesting that PDGFRα may be a marker of resident fibroblasts. The same phenomenon is operative in lungs bearing macro-metastases. In contrast, no BM derived fibroblasts were present in normal mammary glands and normal lungs, indicating active recruitment of BM-derived CAFs into tumors.

Thus, CAFs populations in primary breast tumors and in lung metastases are dynamic in their origin and marker expression which co-evolve with tumor progression.
Osteosarcoma is the most common bone sarcoma in children and adolescents. Metastasis occurs early in the natural history of OS and is a major cause of mortality and morbidity. Virtually all OS patients develop subclinical micro-metastases at initial diagnosis. The rarity and the high genetic heterogeneity of these tumors make it difficult to have patient cohorts that are large enough correlate specific genetic alterations with differences in patient outcome. We have therefore sought to develop well-characterized osteosarcoma derived cell lines to begin to study this problem. In this study, we collected 14 OS cell lines, analyzed their bone marker protein expression in vitro, and characterized their tumorigenicity and the metastatic potential in vivo. To begin to study OS metastasis, six pairs of OS cell lines (four human and two mouse) with high/low metastatic potential were selected and characterized by experimental and spontaneous metastasis experiments. Gene expression profiling of four pairs of high and low metastatic human OS cell lines identified PHLDA1 (TDAG51) as one of the genes more highly expressed in the highly metastatic OS cells comparing to the poorly metastatic OS cells. Down regulation of the extracellular-signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinases (MAPKs)) were observed when knocking down PHLDA1 with siRNA or shRNA. Reducing the expression of TDAG51 also delays OS metastasis progression in mouse xenograft models. Ongoing work is aimed at further delineation of the role of MAPK signaling in metastatic OS and to further understand the role of PHLDA1 in the regulation of MAPKs.
B38 Transketolase a protein regulated by ovarian cancer-peritoneal interactions is elevated in omental metastases and regulates serous ovarian cancer proliferation

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Ovarian cancer, one of the most lethal gynaecological cancers, is characterised by the shedding and dissemination of epithelial cells from the ovarian surface, followed by the spread onto the peritoneal layer surrounding the abdominal organs. A recent proteomic study investigating the interactions between peritoneal (LP-9) cells and metastatic ovarian cancer (OVCAR-5) cells, identified transketolase (TKT) to be upregulated in the conditioned media of ovarian cancer and peritoneal cell co-culture. TKT is a cytosolic enzyme that catalyses key reactions in the non-oxidative branch of the pentose phosphate pathway of glucose metabolism and plays a vital role in de novo ribose generation for nucleotide synthesis in cells. This study characterized TKT expression in stage III/IV serous ovarian cancers (n=137 primary and 50 omental metastases) by immunohistochemistry and evaluated its function in ovarian cancer cells. Nuclear TKT was present in all primary serous ovarian cancer tissues examined (median 81.0 %, range 16.5-100%). Nuclear TKT was significantly increased in omental metastases compared with primary cancers (Mann Witney U test, P=0.001) including 45 matching samples (P=0.03, Wilcoxon Rank test). Kaplan Meier survival and Cox Regression analyses showed that nuclear TKT positivity >90.4% (median level) in the omental metastases was significantly associated with reduced progression-free survival (P=0.022) and a HR of 2.3 (95% CI 1.11-4.88, P=0.026). Knockdown of TKT by siRNA significantly reduced SKOV3 cell proliferation but had no effect on their motility or invasion. Oxythiamine, an inhibitor of TKT activity significantly inhibited the proliferation of invasive ovarian cancer cell lines (OV90, SKOV3, OVCAR-5) and primary serous cancer cells isolated from patient ascites but not the less invasive OVCAR-3 cells. In conclusion, these findings indicate that TKT plays an important role in the proliferation of ovarian cancer cells and could be used as novel therapeutic target for advanced disease.
B39  Role of biglycan in the age-associated malignancy of melanomas

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For the malignant melanoma age has been shown to be an independent adverse prognostic indicator of overall survival. Both, the incidence of cutaneous melanomas as well as the malignancy of the tumor, increase drastically with age. Furthermore, melanomas in young patients grow faster and planar, whereas melanomas in older patients reveal a slow growing however invasive and metastatic phenotype. One possible regulator of this phenomenon is the extracellular matrix of the tumor stroma. Biglycan (BGN), a small leucine rich proteoglycan is a collagen binding constituent of the extracellular matrix. By activation of signaling cascades through e.g. toll like receptors BGN is a known regulator of cellular phenotypes thereby possibly influencing tumor progression and immune responses.

Ex vivo BGN mRNA expression was found to be increased (4.22±0.88 fold of young patients) in fibroblasts derived from old patients (>60 years) compared to young patients (<40 years) as well as in vivo in young (10 weeks) versus old mice (1.5 years). The growth of subcutaneously injected B16F10 melanoma cells in young and old mice was significantly reduced in old mice (0.32±0.13 fold of young mice) resembling the slower progression in old patients as detailed above. In vitro B16F10 melanoma cells were co-cultured with pre-senescent and senescent fibroblasts. Higher expression level of BGN in senescent fibroblasts was correlated with an induction of p21 (3.70±1.28 fold of control) and reduction of proliferation (0.58±0.076 fold of control) in B16F10 cells. Importantly, an increase in AKT2 (1.81±0.28 fold of control), which is associated with increased invasiveness of tumor cells, was found. These effects could be mimicked by treatment of the B16F10 cells with BGN core protein. In addition BGN stimulation induced markers for the epidermal-mesenchymal transition (EMT). EMT has been described as the predominant step in cancer invasiveness and metastasis. Indeed, BGN treatment of B16F10 cells increased the ability for anchorage independent cell growth (2.19±0.54 fold of control) as shown by soft agar assay and invasiveness (2.56±0.188 fold of control) as detected by wound healing assay.

Taken together BGN seemingly modifies the tumor behavior by reducing the growth rate however leading to a more invasive and metastatic phenotype with increasing age.
B40 An integrated systems microscopy and transcriptomics analysis identifies gene signatures of breast cancer cell migratory and invasive behavior

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As breast cancer (BC) mortality results from the development of distant metastases, targeting BC metastasis formation represents an important strategy for the development of improved cancer therapy. A key mechanism driving BC metastasis is the ability of cancer cells to migrate and invade the surrounding tissue. Using a large panel of human breast cancer cell lines we sought to discover gene signatures of BC cell migratory and invasive behavior. We first studied the behavior of 46 different BC cell lines using automated high content epifluorescence microscopy and quantitative image analysis tools. Our results showed that the migration and invasion capacity varies among the different cell lines. In particular, the 2D migration speed was significantly higher in the mesenchymal-like and basal-type cell lines, whereas the basal B cell lines were distinguished as the most invasive in 3D. Next, by integrating transcriptomics data of the BC cell lines we identified the gene expression signatures of the BC cell migratory and invasive phenotypes that comprise a common 440-gene signature. Signaling pathway and protein-protein interaction network analyses of the gene signatures revealed an enrichment for cancer associated genes and signaling networks related to cellular movement and invasion, cell-cell interaction and signaling and cell growth and proliferation. In addition, RNA interference experiments for a total of 170 genes, using three highly motile basal B cell lines, indicated that the gene signatures are also enriched for genes that can functionally regulate cell migration, such as SPEG, VCL, ZEB2, MYH9, KIFC3 and LEPRE1, suggesting a mechanistic relevance in breast cancer metastasis and potential consideration for breast cancer interventions studies. In conclusion, we provide a unique repository of the migratory behavior of a large compendium of human BC cell lines both in 2D and 3D environments, while our computational analysis defines candidate BC metastasis gene sets that likely underlie the aggressive basal-like, triple-negative BC phenotype.
B41 Uncovering the molecular mechanism through which the immediate early gene *Ier2* promotes metastasis

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Metastasis is the major cause of cancer-related mortality. Tumors that have already formed metastases are largely incurable due to their systemic nature which is often accompanied by resistance to therapeutic agents. Thus, there is an urgent need to understand early events of the invasion-metastasis cascade and to identify molecular mechanisms involved in the formation of metastases. In previously published work our group has shown that the immediate early gene *Ier2* promotes tumour cell motility and invasiveness *in vitro* and can stimulate metastasis formation in experimental animals *in vivo*. Furthermore, expression of *Ier2* correlated with poor metastasis-free and overall survival in patients with colorectal adenocarcinomas. These data identify *Ier2* as a novel player in the regulation of tumour progression and metastasis, whose expression is both a prognostic marker and a potential target for cancer therapy. So far, however, the cellular function of *Ier2* is unknown and remains to be resolved. Therefore, we now aim to uncover molecular mechanisms through which *Ier2* exerts its pro-metastatic effects. Specifically, using mass-spectrometry we want to identify interaction partners of *Ier2* to gain insight into the molecular pathways and programs *Ier2* participates in. Having observed that *Ier2* shuttles between the cytoplasm and the nucleus in a cell density dependent-manner, we further plan to investigate if cellular localisation controls activity of the *Ier2* protein and if *Ier2* can regulate target gene expression to shape invasive behaviour of tumour cells.
B42  The Role of Met amplification and Lfng deletion in the development of basal-like breast cancers.

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Basal-like breast tumors are among the most aggressive forms of breast cancer. They are thought to arise from bi-potent progenitor cells in the mammary gland. The Egan lab developed a mouse model for Basal-like breast cancer (BLBC) by altering Notch pathway signalling through deletion of Lunatic Fringe (Lfng). Lfng codes for a sugar transferase that adds GlcNAc sugars to Notch thereby dictating its ligand-binding abilities. Basal-like tumors are commonly hypoxic. Hypoxia leads to gene expression changes that increase chance of survival and this is mediated by the hypoxia inducible factor 1α (HIF1α). Under hypoxia, HIF1α induces hypoxia-responsive genes, including Met and Cav1. Met encodes the Hepatocyte Growth Factor receptor, known for its role in epithelial-to-mesenchymal transition (EMT), which allows cells to migrate and invade other tissues. Cav1 codes for a plasma membrane protein involved in receptor trafficking and signalling. Interestingly, these same genes are co-amplified in our Lfng mutant tumors, suggesting that Met/Cav1 amplification may aid in tumor formation and/or maintenance.

I have conducted immunofluorescence experiments that confirmed the hypoxic status of our tumors. In addition, Met expression was shown to be highly and positively correlated with hypoxia (r= 0.78). Met expression colocalized with markers of luminal (CK8) but not myoepithelial (CK14) mammary cells, and also colocalized with Aldehyde dehydrogenase 1 expression, a marker of stem cells. This data suggest that a hypoxic environment in the tumor may be conducive to higher Met expression in luminal progenitor cells and promote metastatic dissemination in vivo. Our future directions include using the Rosa26 locus to induce overexpression of Met and Cav1 in a Cre-recombinase dependent manner. Using R26Met/Cav1mice we will test for cooperation in tumor development and metastatic dissemination.
B43 Myeloid cell derived vascular endothelial growth factor promotes pulmonary metastasis by increasing endothelial permeability

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Formation of a vascular network is crucial for the malignant progression of solid tumors. The angiogenic drive is caused by increased expression of pro-angiogenic factors, among which the vascular endothelial growth factor (VEGF) is considered to be the most important. Tumor-infiltrating myeloid cells represent a major source of VEGF.

We showed recently that tissue-specific deletion of VEGF in myeloid cells led to a normalized tumor vasculature with increased pericyte coverage, improved oxygenation and accelerated growth of two different tumor models. The tumors also showed enhanced susceptibility to chemotherapeutic treatment. However, it remains unknown whether myeloid cell-derived VEGF also affects invasiveness and metastatic spread of primary tumors and the overall effect of vascular normalization on metastasis formation is also currently under debate. Therefore, we investigated the impact of myeloid cell-derived VEGF on metastasis formation in an autochthonous mouse model of metastasizing breast cancer as well as in subcutaneous isografts of Lewis Lung Carcinoma (LLC).

We found that the loss of VEGF in myeloid cells significantly reduced incidence of metastasis in the isograft model, with a similar trend in the breast cancer model. However the average size of pulmonary metastasis was not affected. Deletion of VEGF in myeloid cells reduced the number of circulating cells after subcutaneous tumor inoculation, and also reduced the number of pulmonary metastasis after intravenous injection of a low number of LLC tumor cells. Ultrastructural analysis by electron microscopy indicate that the VEGF derived from myeloid cells induces a discontinued endothelium of the vascular bed of the lung. The increased permeability of the lung 48 hours after i.v tumor cell injections was confirmed with bronchoalveolar lavage detecting i.v injected Evans blue.

Taken together, these results argue that myeloid cell-derived VEGF is an important regulator of tumor metastasis by facilitating intra- as well as extravasation of tumor cells.
Tumor tissues are unique in the wide range of oxygen concentrations from almost normoxia to anoxia. Hypoxia inducible factor (HIF) is well characterized as an oxygen sensor which plays essential roles in the hypoxic regions of tumor tissues. However, whether HIF also affects tumor malignancy under the moderate hypoxic or normoxic conditions remains unclear. We have identified Mint3 as a HIF activator during normoxia and reported that Mint3 deficient macrophages defect in motility, invasion, and acute cytokine production due to the decreased ATP production via glycolysis during normoxia. Because tumor-associated macrophages (TAMs) promote tumor malignancy, here we examined whether Mint3 in monocytes/macrophages contributes tumor malignancy. Mint3 deficient mice in myeloid cells showed decreased metastasis to the lung compared with control mice. Further analyses revealed that Mint3 in monocytes/macrophages promotes extravasation of cancer cells by inducing E-selectin expression in endothelial cells. Administration of anti-E-selectin neutralizing antibodies also abolished Mint3-mediated tumor metastasis. Thus, Mint3 in monocytes/macrophages is a possible target for preventing cancer metastasis.
B45  Importance of regulation of NMI (N-Myc interactor) in breast cancer progression.

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N-myc interactor (NMI) is an inducible protein that interacts with several transcription factors such as STATs, cMYC, BRCA1, TIP60 and SOX10, all of which have known critical involvement in influencing tumor progression. Our investigations have demonstrated that NMI critically regulates signaling circuitry that negatively influences invasive progression. We find that expression of NMI is down-regulated in breast tumors, especially those displaying invasive features. In breast cancer cell lines, restored expression of NMI suppressed tumor growth. Also, silencing NMI expression from epithelial-like cell lines induced molecular and morphologic attributes of mesenchymal-like phenotype. Mechanistically, loss of NMI had negative impacts on STAT5 driven expression of TGFβ signaling repressor, SMAD7. This downregulation of SMAD7 allowed for aberrant manifestation of TGFβ-driven EMT. Our findings imply that NMI is one of the guardians of the epithelial phenotype. Thus the mechanisms that downregulate NMI expression are of potential interest to understand the etiology of tumor invasion and to identify novel therapeutic targets. We investigated compelling leads to demonstrate that NMI is targeted by miR-29. Luciferase reporter assays supported the projected targeting of NMI by miR-29. Manipulation of miR-29 levels by overexpression or silencing showed its inverse functional relationship with NMI expression. Mesenchymal-like breast cancer cell lines showed elevated endogenous levels of miR-29 relative to non-metastatic (epithelial-like) breast cancer cell lines. More over in contrast to silencing miR-29, transient overexpression of miR-29 enhanced breast cancer cell invasion. The significance of our observation was realized when we compared RNA isolated from patient derived invasive breast tumors and compared with RNA from matched adjacent normal breast tissue. Analysis using the McNemar’s test showed a strong, inverse relationship between the expression of NMI and miR-29. Furthermore our investigations reveal a novel feed forward relationship of miR-29 with loss of NMI. We contend that aberrant miR-29 expression may account for reduced NMI levels and invasive progression in a subset of breast cancers.
B46 Cellular adhesion molecules and brain metastasis; how friends become foes.

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Contemporary anticancer therapies are increasing the patient survival rate for most of tumour types, including breast cancer. As a consequence, patients that survive have a higher risk of developing metastasis. The brain is the only site of tumour relapse in ~60% of lung cancer patients, ~25% of breast cancer patients and ~55% of melanoma patients. Key molecules in this process are cell adhesion molecules (CAMs), which can influence both progression and tumour phenotype as metastases develop. Following initial screening of twelve different CAMs in two different brain metastasis models, we sought to determine the functional role of a subset of these proteins in metastatic seeding and tumour growth.

Human mammary carcinoma (MDA231Br-GFP) and mouse mammary carcinoma (4T1-GFP) cells were injected into female SCID or BALB/c mice, respectively. Two different routes of metastasis induction were used: direct intracerebral injection or intracardial injection to facilitate haematogenous dissemination to the brain. ALCAM, VLA-4 or LFA-1 expression was selectively blocked on the tumour cells prior to injection into the host, using either neutralising antibodies or shRNA gene silencing. Endothelial expression of ALCAM, VCAM-1, E-selectin, ICAM-1, VLA-4 and β4 was markedly increased early in brain metastasis development, whilst their natural ligands were highly expressed on the metastatic cells. Neutralisation of either ALCAM or VLA-4 on the metastatic cells, with blocking antibodies, prior to intracardial injection, significantly reduced the number of metastatic colonies (~60%) within the brain 21 days later, indicating a role for these CAMs in tumour seeding to the brain. Subsequently, we found that knock down of tumour cell LFA-1 gene expression, by shRNA silencing, reduced tumour growth within the brain, with a 75% reduction in tumour volume 14 days after intracerebral injection.

Monoclonal antibody therapies and miRNA approaches make up a significant proportion of current clinical cancer trials. In this study, we have shown that by disrupting the interaction between CAMs expressed on tumour cells and their cognate ligands within the host tissue, it is possible to dramatically reduce metastatic burden in the brain. These data suggest a potential role for CAMs as targets for adjuvant therapies in the battle against brain metastasis.
B47 Genomic and transcriptomic landscapes of human brain metastases


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Brain metastasis is a major unmet clinical need associated with morbidity, neurological decline, virtually 100% mortality and high public health care cost. The prevalence varies between primary cancer types (10-40% overall), developing most frequently in patients with lung, melanoma, renal, breast and colorectal cancers. Brain metastases often develop in heavily pre-treated patients, and commonly recur after neurosurgical resection and/or brain radiotherapy. The etiology and molecular mechanisms underpinning brain metastasis are not well understood. We need to address this knowledge deficiency in order to expand our repertoire of therapeutic options. Unlike primary cancers, metastases have not been extensively studied at the genomic level; particularly brain metastases, which are infrequently resected and rarely available in fresh frozen format suitable for next generation sequencing analysis. We hypothesize that metastatic cells with different evolutionary origins (e.g. primary tumour type, genetic background, clonal selection pressure) engage common mechanisms to exploit the unique brain microenvironment.

In this study we performed whole exome sequencing (Illumina HiSeq) and copy number analysis (Illumina HumanOmni 2.5M SNP arrays) on DNA extracted from a cohort of 36 fresh frozen human brain metastases originating from breast, lung, melanoma and esophageal cancers. RNA was extracted simultaneously with DNA (i.e. from single tissue samples) and also analyzed by HiSeq sequencing. Samples were donated by patients who underwent neurosurgical resection at the Royal Brisbane and Women’s and Gold Coast Hospitals (Queensland, Australia) in the last 5 years. A total of 22,754 somatic single nucleotide variants and small insertion-deletion events (33-3,282/patient) were identified across the cohort. The relative mutation loads and signatures of mutational process (e.g. UV light, smoking, APOBEC) were consistent with expectations based on primary tumour type. Initial analysis of these data has focused on identifying genes and pathways that are recurrently altered across the cohort, which could be involved in the growth or maintenance of brain metastases from different origins. We used a combination of systematic, knowledge- and pathways-based approaches. Key findings will be presented. We hypothesize that analysis and further integration of these data in the future will lead to a better understanding of heterogeneity amongst brain metastases, and ultimately reveal common or recurrent features that could be therapeutically targeted with new approaches, since existing modalities are not broadly effective.

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B48 Exploring the HER3 tyrosine kinase as a therapeutic target for treatment of brain metastases

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Development of brain metastases (BM) is associated with morbidity and neurological decline. BM develop in up to 40% cancer patients. In breast cancer, HER2-positive and triple-negative tumours are most likely to metastasise to the brain. The mortality rate is virtually 100%, with a median survival of 2-22 months depending on treatment and prognostic indicators. There are no standard targeted drug therapies indicated for treatment of BM, though clinical trials in this area are now emerging.

Using immunohistochemical (IHC) analysis of archival tissues, we (Da Silva et al., BCR, 2010) and others (Sun et al., CCR, 2008) demonstrated that HER3 (ERBB3) is induced in BM compared to matching primary breast and lung cancers, suggesting it could be beneficially activated in the neuregulin (Nrg)-rich brain microenvironment. In support of a paracrine mode of activation, we detected NRG1 and NRG2 transcripts at very low relative abundance in BM by RNAseq analysis, and could suppress growth of intracranial MDA-MB-231 breast cancer xenografts by co-grafting a neutralizing Nrg-1 antibody. Xenograft growth was also suppressed in mice treated with Herceptin (i.p.). We found ERBB3 RNA levels correlate strongly with ERBB2 in clinical samples, but at the protein level HER3 is ubiquitously activated (phosphorylated) in cases comprising a range of HER2 activation levels. IHC analysis of a large archival BM cohort (n=170; 7 primary cancer types) showed strong, complete membrane staining of pHER3 in 57.7% cases. We also analysed expression and activation of EGFR, HER2 and HER4 in this series. Collectively these data suggest that targeting HER3 could be a good therapeutic strategy.

To begin to investigate the mechanisms by which HER3 may promote the growth of BM from breast cancer, we studied breast cancer cell lines treated with recombinant Nrg-1 (heregulin). We found that Nrg-1 induces phosphorylation cascades involving HER2, HER3, Akt and ERK 1/2 (but not HER4), induces expression of p27 and Cyclin D1, and increases proliferative and migratory behavior in MCF7, SKBr3 and MDA-MB-361 breast cancer cell lines. In addition to these classical pro-tumourigenic activities, we also tested whether canonical HER3 signaling can promote extracellular protease activity, since protease-mediated modification of the tumour microenvironment is thought to be beneficial in some contexts (e.g. increasing growth factor availability, permeabilising the blood-brain-barrier). Nrg-1 treatment increased the expression and activities of Cathepsin B, MMP-2 and MMP-9 in HER2/3+ breast cancer cell lines, and permeabilised a tight human brain microvascular endothelial cell layer in vitro. Treatment with GM6001 (broad spectrum MMP inhibitor), Herceptin or the humanized HER3 monoclonal antibody EV20 opposed Nrg-1-mediated permeability in this assay.
B49  Small hyaluronan oligosaccharides: novel regulators of lymphangiogenesis and metastasis

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Hyaluronic acid (HA) is composed of repeating disaccharide units of D-glucuronate and N-acetylglucosamine, and is a major component of the extracellular matrix. Within tissues it normally exists as a high molecular weight polymer and is synthesized and accumulated by most cells, particularly during proliferation. HA turnover occurs constantly, but is enhanced in tumors and in areas of wounding and inflammation through the activation of endogenous hyaluronidases and also by reactive oxygen species, processes that degrade high molecular weight HA into small fragments. The small HA oligosaccharides (sHA) so produced are highly bioactive and proinflammatory. A major role for sHA, particularly in the context of tumors, is in the induction of angiogenesis. Thus, while high molecular weight HA is anti-angiogenic, sHA ranging in size from 3 to 25 disaccharides in length is a potent inducer of angiogenesis. Here we report that sHA also promotes lymphangiogenesis, as at concentrations of 1-10 µg/ml it stimulates the proliferation of primary lymphatic endothelial cells (LECs) and induces outgrowth of lymphatic capillaries in ex vivo thoracic duct ring assays. Lymphatic vessel density in lymph nodes draining skin into which sHA has been intradermally injected is also increased. VEGF-C and sHA act additively to induce lymphangiogenesis. Loss of function analysis indicates that LYVE-1 is the receptor that mediates the pro-lymphangiogenic role of sHA. At concentrations above 10 µg/ml, sHA progressively inhibits LEC proliferation and lymphangiogenesis, and induces an endothelial-mesenchymal transition in which expression of lymphatic markers is lost and LECs take on a mesenchymal phenotype. At least part of this effect is mediated by TGFβ, whose expression in LECs increases in response to sHA in a dose-dependent manner. Tumor interstitial fluid (TIF) can contain up to 6 µg/ml sHA, indicating that sHA accumulation in tumors may contribute to tumor-induced lymphangiogenesis. Consistently, we found that enhanced levels of sHA in TIF from colorectal cancer patients correlates with lymph node metastasis and invasion of tumor cells into lymphatic vessels.
B50 An endothelial-mesenchymal transition of brain endothelial cells is required for metastatic extravasation

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Endothelial-mesenchymal transition (EndMT) is an embryonic program necessary for organ development. Despite being normally dormant in adult organisms, this mechanism can be reactivated during several pathological conditions, such as cancer and fibrosis. EndMT is characterized by the downregulation of the endothelial program, activation of the mesenchymal-fibrogenic program, and finally the activation of the myogenic program. Cancer progression towards metastasis follows a defined sequence of events described as the metastatic cascade. For extravasation and transendothelial migration metastatic cells interact first with endothelial cells. Yet the role of endothelial cells during the process of metastasis formation and extravasation is still unclear, and the interaction between metastatic and endothelial cells during transendothelial migration is poorly understood. Since tumor cells are well known to express TGF-β, and the compact endothelial layer undergoes a series of changes during metastatic extravasation (cell contact disruption, cytoskeletal reorganization, contractility), we hypothesized that an EndMT may be necessary for metastatic extravasation.

We show that primary rat brain endothelial cells (BECs) undergo EndMT upon TGF-β1 treatment, characterized by the loss of tight and adherent junction proteins, the expression of fibronectin, β1-integrin, calponin and α-smooth muscle actin (SMA), and an increase in N-cadherin levels. B16/F10 activated conditioned medium (ACM) induced claudin-5 downregulation, fibronectin and SMA expression in BECs. Inhibition of TGF-β signaling during B16/F10 ACM stimulation using SB-431542 maintained claudin-5 levels and mitigated fibronectin and SMA expression. Moreover, SB-431542 prevented SMA upregulation upon stimulation of BECs with A2058, MCF-7 and MDA-MB231 ACM. Moreover, B16/F10 ACM caused a reduction in transendothelial electrical resistance and enhanced the number of melanoma cells adhering to the endothelial layer, both in a TGF-β dependent manner. These effects were not confined to BECs, since HUVECs also showed TGF-β dependent SMA expression when stimulated with breast cancer cell line ACM.

Our results indicate that an EndMT may be necessary for metastatic transendothelial migration, and may play a relevant role during the complex phenomenon known as metastatic extravasation.
B51  In Vivo Image-based Quantitation of Circulating Tumor Cells by Real-time Video-rate Confocal Microscopy

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The number of circulating tumor cells (CTCs) in the blood of metastatic cancer patients can be a valuable indicator for prognosis. It can be utilized for a metastasis grading, efficacy assessment of anti-cancer therapy, and potentially for early detection of recurrent cancer. Currently, the major strategy for CTC quantitation is using either a microfluidic chip or magnetic beads to isolate and detect CTCs from ex vivo blood sample drawn from the patient. However, these ex vivo quantitation approaches using very small volume of blood sample (typically 5-10 ml) is technically challenging due to the extreme rareness of CTCs (<1 CTC per ml of blood), which results in low sensitivity and high false negative error. In vivo quantitation of CTC directly from blood vessel is an attractive approach to improve the detection sensitivity by analyzing significantly larger volumes of continuously flowing blood. In this study, we implemented a custom-design, video-rate intravital confocal microscopy system that can directly image freely flowing CTC in the blood vessel of mouse model. Based on fast-rotating polygonal mirror scanner, it can acquire three-color fluorescence confocal images at 100 frames/sec (512x512 pixels), which allowed us direct imaging of rapidly flowing (~6 mm/sec) individual cells in the great saphenous vein (GSV) of a mouse model in vivo. After intravenous injection of CT26 colorectal cancer cells and red blood cells (RBCs) fluorescently labeled with CFSE (green) and DiD (far-red) respectively, we successfully achieved clear imaging of fast circulating CT26 cells and RBCs over several hours. The number of flowing CT26 cells reached the maximum peak value right after the IV injection and decreased below 10% of peak value within 3 minutes at GSV. Interestingly, we observed huge amounts of cellular debris fluorescent in green as well, which might be the remnant of broken CT26 cells due to the harsh condition of blood circulation. For accurate quantitation of intact CT26 cells distinguished from cellular debris, we developed an image-processing algorithm that count only cell-sized object. On the other hand, the number of flowing RBCs remained at same initial value over 60 minutes. As the total number of injected RBCs circulating in whole body blood is pre-determined, we could calculate a calibration factor based on the number of RBCs detected by the imaging system to relatively quantitate any other circulating cells including CTCs in whole body blood in vivo. Furthermore, as the in vivo imaging procedure doesn’t require ex vivo blood sampling, the quantitation of CTCs can be repeated on single mouse indefinitely. We repeatedly monitor the number of CTCs at the GSV of various type of tumor mice model over 6 weeks, allowing us longitudinal observation of CTC number disseminated from the implanted primary tumor along with its growth and distant organ metastasis.
B52  Tumor Cell-Secreted Osteopontin Activates Normal Mammary Fibroblasts to acquire a CAF-like phenotype.

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Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Cancer Associated Fibroblasts (CAFs) are an activated sub-population of stromal fibroblasts, which can have different characteristics in different tumor types. CAFs were shown to promote tumor growth by directly stimulating tumor cell proliferation and by enhancing angiogenesis and inflammation. We demonstrated that normal fibroblasts can be reprogrammed by tumor cells to become activated, pro-inflammatory CAFs. In an effort to identify tumor-derived factors that are capable of reprogramming normal mammary fibroblasts to CAFs, we performed a proteomics analysis of tumor cell-secreted factors and identified Osteopontin (OPN), highly secreted by mouse and human breast cancer cells. Osteopontin (OPN) is a secreted phosphorylated glycoprotein, implicated in inflammation, tumor progression and metastasis. We show that breast tumor cell-derived OPN can functionally activate a pro-inflammatory gene expression signature and enhanced motility in normal mammary fibroblasts (NMFs), characteristic of CAFs. Recombinant OPN was sufficient to induce reprogramming of NMFs and neutralizing antibodies to OPN could block NMF activation by tumor cell-conditioned medium. Furthermore, we show that activation of mammary fibroblasts by secreted OPN depends on both its known promoters: CD44 and α₅β₃. Taken together, we demonstrate a functional role for paracrine signaling by tumor-derived OPN in reprogramming NMFs to become pro-inflammatory CAFs.
B53 Cellular crosstalk mediated by Hedgehog signaling in the tumor microenvironment

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Aberrant activation of the Hedgehog (Hh) signaling pathway is a salient feature of many malignancies. Besides pathway mutations, activation of Hh signaling can be autocrine or paracrine. We hypothesized that breast cancer cells influence other cells in their microenvironment by paracrine activation of Hh signaling in cells comprising the milieu at the primary tumor site as well at the metastatic site. Sonic Hedgehog (SHH)-mediated signaling initiates a cascade of signaling events that culminates in the translocation of activated GLI transcription factors to the nucleus to regulate target gene transcription. We modulated Hh signaling in tumor cells and identified the oncoprotein, osteopontin and the cysteine-rich angiogenic inducer, CYR61 as the major proteins in the secretome of the tumor cells. Both proteins can signal through multiple cell surface receptors. Detailed molecular and functional analyses allowed us to assign important roles for osteopontin and CYR61 in mediating the pro-malignant effects of Hh signaling.

Breast cancer has a high propensity to metastasize to bone; nearly 60-75% of breast cancer patients develop bone metastases. GLI1 silencing decreased mesenchymal characteristics of tumor cells and also significantly decreased the intensity and incidence of osteolytic bone metastases. We characterized the nature of paracrine Hh signaling between breast cancer cells and osteoclasts and osteoblasts, the predominant cell types in bone. We determined that Hh ligands produced by breast cancer cells cause paracrine activation of Hh signaling in pre-osteoclasts consequently upregulating osteoclastogenesis (TRAP staining) and resorptive activity characterized by increased levels of the essential proteases, cathepsin K and MMP9. We further noted that inhibition of Hh signaling in breast cancer cells resulted in decreased tumor biomass in the femur and tibia of athymic mice injected with breast cancer cells. Cumulatively our studies suggest that breast cancer cells orchestrate a chain of events, via Hh signaling, in which they directly foster osteolytic bone metastasis. The hypoxic environment in bone (pO$_2$ between 1–7%) imposes cells to adapt their molecular functions to respond to the hypoxic niche. We hypothesized that the hypoxic environment in bone exercises an adaptive response in breast cancer cells characterized by an altered profile of Hh-responsive genes. This altered expression profile mediated by the GLI transcription factors enhances the osteolytic activity of breast cancer cells. Utilizing a strategy of inhibiting GLI transcriptional activity in combination with normoxic or hypoxic conditions we have investigated the molecular re-programming of Hh signaling that results in an altered GLI transcriptome. As such, our work addresses the tumor microenvironment conditions (hypoxia) that alter the transcriptional activity of the GLI transcription factors of the Hh pathway. The identification of novel candidates of Hh signaling will identify putative therapeutic targets to intervene in osteolytic metastasis of breast cancer, and identify molecular surrogates that serve to indicate the efficacy of inhibiting Hh signaling in the hypoxic bone environment.
FN14 expression in breast cancer is a prognostic and predictive biomarker of brain metastasis outcome that can be targeted with immunomodulators

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Brain metastases have become a significant clinical problem, with an incidence of 30-40%, affecting particularly those patients whose tumors over-express ErbB2/HER2, which increases brain colonization 2.5-fold to 3-fold, and patients with triple negative tumors (hormone receptor–ER and PR negative and ErbB2 negative). We have previously reported brain metastasis biomarkers to prediction/prognosis when are present in primary breast carcinomas. To further validate them, we performed a multicenter analysis and immunohistochemistry analysis was established in tissue macro arrays from 318 patients with breast carcinomas (from years 1989 to 2009), which had 138 patients with metastasis, among them 84 had brain metastasis. GRP94 and FN14 levels showed a significant correlation with brain metastasis progression ($p=0.0003$ and $p<0.0001$, respectively); the likelihood to develop brain metastasis increased 2.55 (95%CI 1.52 - 4.3) and 5.24 (95%CI 2.83 - 9.71) folds, respectively. The model that combined GRP94 and FN14 expression further increased the discrimination of brain metastasis development risk (AUC=0.69) with regard to ErbB2 (AUC=0.57). Moreover, FN14 had more sensibility than ErbB2 (38.27 vs. 24.68) with similar specificity (89,43 vs. 89,55) to predict brain metastasis and identical prognostic value than triple negative patients (N=81 and N=77, respectively, $p<0.0001$). Furthermore, we used a protein-protein interaction network based on GRP94 and FN14 pathways and GUILD, a network-based disease-gene prioritization program, to pinpoint the genes that are more likely to drive therapies due to their location in the network. The main modulator was FN14 able to be targeted with thalidomide. Lenalidomide, a derivate from thalidomide with increased immunomodulatory activity and anti-inflammatory properties were tested in preclinical brain metastasis models resulting in an improvement of mice survival, particularly when the treatment is coupled with an inhibitor of GRP94, validating the efficacy of them to improve the outcome of brain metastasis. In conclusion, FN14 and GRP94 expression in breast cancer is opening new possibilities to prevent/treat brain metastasis.

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Determination of Novel Molecular Pathways Regulated by Kallikrein-related Peptidase 7 in Ovarian Cancer

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Kallikrein-related peptidase (KLK) 7 is a serine protease that is over-expressed in ovarian cancer cells, compared to normal ovarian tissues. Although KLK7 function is predominantly proteolytic, only a few substrates have been identified in a biochemical context, which has linked KLK7 to tumour progression. However a proteome-wide study to define the KLK7 biological function in this cancer is long overdue. Hence, we sought to define the substrate mediators and the down-stream gene targets of KLK7, employing a subsite centric degradomics technique and a high-throughput cDNA sequencing technique (RNA-Seq), respectively.

Proteomic Identification of protease Cleavage Sites (PICS) employs enzymatic cleavage of trypsin/GluC/chymotrypsin–derived peptide libraries with recombinant active KLK7 and a double mutant (dm)KLK7 (D112N and S205A in the catalytic triad) as control, and liquid chromatography (LC)-MS/MS analysis to identify the peptide fragments generated by KLK7 followed by a bioinformatics-based extension that accounts for structural availability. Furthermore, KLK7 and dmKLK7 treated SKOV-3 cell RNA was sequenced to identify the pathways affected downstream of KLK7 proteolytic activity.

Thirty putative KLK7 substrates were identified along with 3 known substrates, validating our proteomics technique. The majority were novel substrates, including extra cellular matrix components, growth factors, cytokines and cell surface receptors, degradation of which is associated with increased tumour progression. Importantly, Transcriptome Sequencing data showed changes in gene expression level upon KLK7 treatment, suggesting a role of KLK7 in rendering the tumour microenvironment permissive for cancer progression.

This study has identified novel molecular pathways by which KLK7 may function to promote the progression of ovarian cancer. Novel molecular pathways identified through this study will provide impetus for the development of biologically consequential targets for new therapeutics.
B56 The role of epithelial to mesenchymal plasticity in driving the invasive capacity of ‘lobular-like’ tumour cell differentiation in breast cancer.

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Epithelial to mesenchymal plasticity (EMP) is implicated as an important process in tumour progression, contributing to an enhanced tumour cell invasive and metastatic capability. Evidence for EMP occurring in breast cancer has come from studies of the aggressive subtype of high-grade invasive carcinomas with basal-like features. These tumours show the highest frequency of cadherin-switching (reduced E-cadherin and activated N-cadherin expression) and the activation of mesenchymal markers (e.g. smooth muscle actin and vimentin). Invasive lobular carcinomas are less aggressive in the short term but they exhibit a very invasive growth pattern and are highly metastatic in some patients and consistently exhibit dysfunctional cell-cell adhesion through loss of E-cadherin. The role EMP plays in lobular tumourigenesis is controversial in the literature1-3, but the underlying hypothesis from the analysis of small numbers of samples, is that it EMP is an unlikely phenomenon.

We have examined a series of markers of EMP in breast cancer samples showing a lobular-like growth pattern, including a large series of invasive lobular carcinomas and a series of mixed tumours in which there is morphological evidence of a transition from ‘ductal’ (tumour nests) to regions of neoplastic cells showing lobular-like differentiation (single files of cells or single cells) with enhanced invasive capacity. From a cohort of 148 ILC we observe infrequent evidence of expression of mesenchymal markers in tumour epithelial cells and little change in phenotype during progression to lymph node metastases in 37 of these cases. In 3 cases however there was concomitant expression of TWIST (nuclear localization), vimentin, smooth muscle actin and osteonectin implying a partial EMP might contribute to the invasive nature of these individual tumours. No evidence was seen for EMP in the progression of ductal to lobular like patterns of growth in mixed tumours or in a case that showed transition from a primary ductal carcinoma to a gastric metastasis exhibiting a diffuse lobular growth pattern. Snail nuclear localization was only ever evident in these cases in areas associated with necrosis.

In summary, we rarely observe evidence of EMP in clinical samples showing lobular carcinoma-like differentiation, neither in classic invasive lobular carcinomas nor in mixed tumours that show transition from ductal to lobular-like growth pattern. This data supports the notion that lobular carcinomas are epithelial in nature and that E-cadherin down-regulation / dysfunction occurs by other means.

References
NR2F1 controls tumor cell fate via SOX9 and RARβ driven quiescence and pluripotency programs.

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We identify the orphan nuclear receptor NR2F1 as a fate determinant in disseminated tumor cells (DTCs). NR2F1 is frequently epigenetically upregulated in quiescence tumor cells in experimental models and in DTCs from PCa patients carrying dormant disease for 7-18 years. Dormancy was recapitulated by a co-treatment with the DNA demethylating agent 5-Aza-C followed by retinoic acid across various cancer types. NR2F1-induced quiescence was dependent on SOX9, RARβ and CDK inhibitors. Intriguingly, NR2F1 induces global chromatin repression and the pluripotency genes NANOG and SOX2, suggesting tumor cells can commit to a stable but reversible G0-arrest and latent pluripotency programs via NR2F1. When NR2F1 is blocked in vivo, microenvironment-specific dormancy or survival of occult DTCs is interrupted. The ability of NR2F1 to stably commit tumor cells to quiescence, survival and latent pluripotency may explain their ability to interconvert between dormancy and proliferation. We propose that NR2F1 may mark non-proliferative DTCs with the capacity to survive and eventually escape dormancy.
The extracellular matrix protein Nephronectin promotes metastasis via its integrin binding domain

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Cellular interaction with the tumor microenvironment is an important feature of tumor progression. We have previously demonstrated that the extracellular matrix (ECM) protein Nephronectin (Npnt) promotes metastasis in a mouse model of breast cancer. In the current study, we have characterized the expression of Npnt in various human and mouse breast cancer model systems. Using the MMTV-PyMT transgenic mouse model, where the tumor progression is driven by the Polyoma Middle T oncoprotein, and where palpable mammary tumors arise spontaneously within 5 weeks of birth, we find that Npnt is expressed at high levels at both the carcinoma in situ stage as well as in invasive carcinomas. Furthermore, we show that Npnt is expressed in human MCF-7, SKBR3, BT474 and MDA-MB231 xenografted tumors, indicating a potential role in human breast cancer.

To understand the mechanism by which Npnt promotes metastasis, we have generated mutant cell lines stably expressing either the wild type Npnt protein or mutant Npnt with disrupted integrin binding sites. We created a mutation in the RGD integrin binding site and a double mutation that disrupted both the RGD and the integrin binding enhancer site EIE and expressed these constructs in the 66cl4 low metastatic mammary tumor line. An in vivo metastasis assay demonstrated that 66cl4-Npnt cells have a significantly increased ability to metastasize and that the integrin binding site double mutation prevents this enhancement of metastasis. Taken together, our findings show that the extracellular matrix protein Npnt promotes metastasis and this is mediated via the integrin binding (RGD) and integrin binding enhancer (EIE) sites.
**B59 Regulation of Pro-Inflammatory and Pro-Angiogenic Cytokines in Breast and Lung Cancer Cells: Implication of Epithelial-to-Mesenchymal Transitions.**

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Increasing data suggest an implication of epithelial-to-mesenchymal transitions (EMT) in breast cancer progression. Markers of EMT have indeed been observed in breast tumours and are associated with lymph node metastasis, high histological grade and poor prognosis.

We hypothesized that EMT programs might alter the cytokinic microenvironment and thereby stimulate host cells recruitment, which would favour angiogenesis and inflammation, thereby facilitating tumour progression.

To test this hypothesis, we used two human cell lines, MDA-MB-468 (mammary adenocarcinoma) and A549 (lung carcinoma), that were shown to undergo EMT changes following EGF and TGF-beta treatment respectively. A cytokine array on cell supernatant showed that EMT induction in these cell lines is associated with the upregulation of a consistent panel of cytokines. We indeed confirmed by RT-qPCR and ELISA that Interleukin-8 (IL-8), Interleukin-6 (IL-6), Granulocyte Monocyte-Colony Stimulating Factor (GM-CSF), soluble InterCellular Adhesion Molecule 1 (sICAM-1) and Plasminogen Activator Inhibitor (PAI-1) are upregulated during EMT in the two cell lines.

More particularly involving EMT in such regulation, we observed that the modulation of two major EMT transcription factors (Snail and Slug) by siRNA or cDNA transfection regulated cytokine expression. Inversely, ectopic expression of a stabilized form of Snail was sufficient to significantly increase expression of all studied cytokines.

In addition, conditioned medium from EMT+ MDA-MB-468 cells increased the density of blood vessels in an in vivo ‘Sponge Assay’ and increased blood vessel sprouting from rat aortic rings in vitro, suggesting a functional effect of these cytokines on the vascular network.

Moreover, preliminary in vivo data suggest that conditioned medium from EMT-derived cells is able to significantly increase the recruitment of myeloid-derived suppressor cells (MDSC) in vivo. MDSC are a heterogeneous population of immature myeloid cells that all display an immunosuppressive function.

In summary, our data demonstrate that EMT is associated with an overexpression of a panel of cytokines and imply that snail might be sufficient for their expression. At last, our results suggest that EMT-induced cytokines could favour tumour-associated angiogenesis and the recruitment of MDSC, thereby facilitating the metastatic progression.
B60   The promotion of breast cancer metastasis caused by inhibition of CSF-1R/CSF-1 signaling is blocked by targeting the G-CSF receptor

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Treatment options are limited for breast cancer patients presenting with metastatic disease. Targeting of tumor-associated macrophages through inhibition of colony stimulating factor-1 receptor (CSF-1R), a key macrophage signaling pathway, has been reported to reduce tumor growth and metastasis, and these treatments are now in clinical trials. Here we report that, surprisingly, treatment with neutralizing anti-CSF-1R and anti-CSF-1 antibodies, or with two different small molecule inhibitors of CSF-1R, could actually increase spontaneous metastasis, without altering primary tumor growth in mice bearing two independently derived mammary tumors. The blockade of CSF-1R or CSF-1 led to increased serum G-CSF levels, increased neutrophils in the primary tumor and in the metastasis-associated lung, as well as increased neutrophils and Ly6Chi monocytes in peripheral blood. Neutralizing antibody against the granulocyte-colony stimulating factor receptor (G-CSFR), a key receptor controlling neutrophil development and function, reduced the enhanced metastasis and neutrophil numbers seen after CSF-1R blockade. These results indicate that the role of the CSF-1R/CSF-1 system in breast cancer is far more complex than originally proposed, and requires further investigation as a therapeutic target.
B61  Diets high in saturated fats stimulate metastasis of orthotopic mammary tumors to the brain and bone by orthotopic mammary tumors


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Diets containing high levels of fat have been suggested to induce low-grade inflammation in association with hematopoietic progenitor cell mobilization. In our studies, we compared Lieber-DeCarli liquid diets containing either 36% or 12.5% of calories as fat, to animals on a chow fed diet with 16% of calories as fat. The Lieber-DeCarli diets have a higher saturated fat content as compared to the chow fed diet. In our studies, mice fed the Lieber-DeCarli diets had increased inflammation including hepatic and splenic extramedullary myelopoiesis (EMM) as assessed by flow cytometry, immunohistochemistry (IHC) and a colony forming unit-granulocyte macrophage (CFU-GM) assay. Further, an increased number of myeloid derived suppressor cells (MDSCs) CD11b^Gr1^ cells that were predominantly Ly6G^br^ were observed in the liver, spleen and bone marrow (BM). The increase in hepatic MDSCs was associated with an increased number of adipocytes (Oil Red O) and Gr1^+^ cells as determined by immunohistochemistry. Consistent with the increased number of hepatic MDSCs and EMM is a decrease in BM cellularity and progenitor cells as measured by flow cytometry (Lin^-^CD11b^-^Gr1^-^Sca^-^1^+) and CFU-GM/femur. We posit that the low grade, chronic inflammation associated with the Lieber-DeCarli diets contributed to the observations of a shortened time to primary tumor induction of the orthotopic 4T1 mammary tumors and increased numbers and metastatic sites including splenic, BM, cardiac, hepatic, renal, ovarian, brain and extensive lymph node foci in addition to peritoneal and pleural effusions. Unexpectedly, in mice bearing an unselected 4T1 tumor cell population growing at an orthotropic site; brain metastases (<1 mm diameter) were observed supporting a regulatory role for saturated fat in tumor progression and metastasis. Further, in the tumor bearing mice consuming the high fat Lieber-DeCarli diet we observed extensive cortical and trabecular demineralization and bone loss by micro computed tomography (micro CT) including femur, tibia and vertebral column that was associated with TRAP^+^ osteoclast activity. This bone loss was most notable in the distal femur and proximal tibia and associated with osteoelast channels, and appeared to be associated with areas of active myelopoiesis. The latter were haemorrhagic with a predominant nucleated cell infiltrate composed of bands, segs and myelocytes compatible with EMM. These results suggest that a diet high in saturated fats can increase tumor induction, metastasis and pathology in association with MDSC mobilization, expansion and EMM resulting in increased numbers of osteoclasts, associated bone demineralization and suppression of T-cell frequency and function. These results support a need to develop clinical, combination, therapeutic strategies incorporating components that inhibit OCs, MDSCs and their associated mediators. Furthermore, we suggest the potential for dietary manipulation to regulate primary tumor growth and sites of metastasis and that these studies provide a highly clinically relevant model of spontaneous mammary tumor metastasis to bones and brain.
Intravital imaging of organ colonization by circulating melanoma cells: steps and strategies of individual cells versus tumor cell clusters

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Cancer metastasis by the hematogenous route is a multi-step process including local cancer invasion, intravasation, intravascular survival, extravasation, and metastatic colonization. Besides single cells, clustered (2-50 cells) circulating tumor cells (CTCs) can be detected in peripheral blood of cancer patients. Whereas clusters have been shown to have a higher metastatic capacity in vivo, the arrival and extravasation process itself as well as underlying mechanisms of relative survival properties of single vs clustered CTCs remain unexplored. In contrast to later phases of metastatic outgrowth, the steps and mechanisms of early steps of organ colonization including biomechanical challenge and the routes taken by single versus clustered CTCs remain unclear.

We here established a skin-homing metastasis model in living mice to monitor initial steps of metastatic seeding by long-term intravital microscopy. For optical access to dermal blood vessels, a skin-fold chamber was transplanted on the back of an immunocompetent mouse and metastatic single and clustered murine B16F10 melanoma cells injected into the left ventricle. CTC arrival in the skin was monitored by epifluorescence and real-time intravital multiphoton microscopy and long-term metastatic colonization to skin and other organs (lung, liver and brain) detected by epifluorescence microscopy post-mortem up to 4 weeks post-tumor cell injection.

With this model, the following steps of early organ colonization were observed: CTCs circulating in the blood stream (capillary slipping, arteriole passage and rolling); locations of intravascular arrest, adhesion and spreading; intravascular survival challenges (tumor cell fragmentation, apoptotic nuclei); the process of extravasation including cell and nuclear deformation and perivascular spreading; as well as metastatic outgrowth and invasion for single and clustered CTCs resulting in microcolonies of varying size. In detail, intravascular arrest of single-cell and clustered CTCs occurred in small and mid-sized vessels by either vessel embolization and occlusion or lateral adhesion and elongation dependent on vessel size. Single-cell extravasation was a slow process, with duration up to 12 hrs, and characterized by strong nuclear deformation (diameter < 1um) and oscillating pore-transmigration kinetics. In contrast to single cells, CTC clusters extravasated in pearl-chain like configuration through the same pore with little nuclear deformation and higher speed (< 4 hrs), indicating extravasation “hot spots”. In conclusion, we established a robust model for high-resolution intravital microscopy enabling to explore seeding locations, survival and extravasation mechanisms as well as rate-limiting steps for survival and outgrowth for single versus CTC clusters.
B63 Integrated Target Discovery in the EMPathy Breast Cancer Network - Multidimensional Analysis of Epithelial Mesenchymal Plasticity (EMP) in Experimental Systems.

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The ability of breast cancer cells to switch between epithelial and mesenchymal phenotypes may be key to their survival in new environments, resistance to therapies and ability to form metastases. Epithelial mesenchymal plasticity (EMP) is instrumental in embryological development and has been implicated in stemness, therapy resistance and metastasis of breast cancer. EMP markers are enriched in basal-like, triple negative breast cancer, which is a type of breast cancer associated with early recurrence and poor prognosis, and established as a common phenotype in women with BRCA1 mutations. The EMPathy Breast Cancer Network (BCN) is a national collaborative effort including scientists, surgeons, medical oncologists and a consumer advocate investigating the role of EMP in breast cancer recurrence. The 7th thematic research projects of EMPathy BCN, including the 9 program-funded ‘Satellite’ projects, are aligned with the Cooperative Research Centre for Cancer Therapeutics (CTx) (www.cancercrc.com/index), so that any potential drug targets identified may progress into the CTx drug development program.

Multiple parallel approaches in the Target Discovery theme were used to identify candidate regulators and effectors of EMP. A total of 10 functional or gene expression experiments provided 7,950 significant events in any one system, which were cross referenced against 10 public breast cancer datasets relevant to EMP and/or breast cancer stem cells. A series of criteria were used to select a panel of 127 candidates that were combined with 123 ad hoc candidates (mainly hits close to the cut-off and breast cancer context genes) to give a total of 250 candidates to be analysed in breast cancer tissues using Nanostring technology. The 2,301 ‘significant events’ in any functional screen were further cross-referenced to 10 public functional datasets relevant to EMP in any system and a series of criteria were used to select a panel of 320 candidates that are to be analysed in an siRNA ‘functional screen’ of multiple breast cancer cell lines, to support the choice of Candidate Targets for drug development. Ongoing studies will address the biology behind selected Candidates.

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B64  An *in vivo* shRNA screen for Breast Cancer Metastasis Suppressor Genes

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When diagnosed early, breast cancer can be treated with a high degree of success. However, once the disease reaches the metastatic stage, and the cancer spreads to distant organs such as lungs and bones, the treatments are extremely limited and the prognosis is very poor. By identifying genes that control metastasis, it will be possible to predict the patients whose disease is likely to spread and also create an opportunity to develop more efficient therapies. Using a lentiviral shRNA library, we initiated a genome-wide *in vivo* screen to identify genes whose reduction in expression levels in weakly metastatic or non-metastatic mouse mammary tumour cell lines lead to spontaneous metastasis to secondary sites. After five screens using four of ten subpools contained in this library, we found 90 unusual metastases in organs such as bones, brain, lungs, liver, ovary, heart, skeletal muscle, kidney, lymph node, contralateral mammary fat pad and adrenal gland. Dozens of putative candidates were identified, including some genes which are either (1) significantly diminished at the DNA and transcript levels in aggressive human breast carcinomas versus normal breast tissue, and/or their underexpression is associated with poor prognosis in breast cancer (Oncomine) or (2) have a suggestive biological role, presented a strong phenotype during the screen and are downregulated in more aggressive breast cancer cell lines. These candidates are now under additional analysis to be validated as *bona fide* metastasis suppressor genes.
B65  Identification and validation of two novel metastatic markers in Osteosarcoma

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Background:
Osteosarcoma (OS) accounts for 56% of malignant bone cancers in children and adolescents. Pulmonary metastasis occurs in approximately 50% of patients and leads to a 5-year survival rate of less than 20%. Patients with metastatic disease are refractory to current chemotherapeutic treatments. Therefore, it is crucial to identify genes and pathways that drive the metastatic behavior of OS in order to find new effective therapeutic targets.

Methods:
To identify markers that may define inherent metastatic behaviour in OS, we conducted microarray-based comparative profiling analysis of clonal variants from an inherently metastatic cell line, KHOS. Two highly metastatic (C1 and C6) and two poorly metastatic clonal variants (C4 and C5) were compared in the transcriptomic screen.

Results:
DCBLD2 and TXNRD2 were identified as potential markers for OS metastasis with 2-4 fold increased expression in the highly metastatic clonal variants. Gene expression of DCBLD2 and TXNRD2 was validated in a transcriptomic screen of non-malignant bone (NB), and chemo-naïve biopsies of OS patients with metastatic disease (M-OS), or localized disease (NM-OS). These markers were found to be highly expressed in 29-42% of M-OS with little to no expression seen in NB and NM-OS. Knockdown of DCBLD2 using shRNA showed a significant decrease in pulmonary metastasis in vivo. Targeting TXNRD2 with a commercially available drug specific inhibitor, auranofin, significantly reduced migration and invasion in vitro, and significantly decreased pulmonary metastasis in a mouse model of spontaneous OS lung metastasis.

Conclusions:
This transcriptomic screen identified TXNRD2 and DCBLD2 as promising targets for the prevention and treatment of metastatic OS.
R-spondin1 functions are regulated by N-glycosylation

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Introduction: The Wnt pathway agonist R-spondin1 is a secreted protein expected to a therapeutic application [1,2]. R-spondin1 has been proven to have proliferative effects on intestinal crypt cells [1]. Therefore, R-spondin1 has been evaluated with promising results to act as therapeutic adjuvants by enhancement of host tolerance to aggressive chemoradiotherapy for eradicating metastatic cancers [2]. R-spondin1 contains one consensus sequence for N-glycosylation (Asn-Xaa-Ser/Thr: Xaa represents any amino acid except Pro) at Asn\(^{137}\), but it remains unknown whether R-spondin1 is N-glycosylated or not. Here we show that R-spondin1 is N-glycosylated at Asn\(^{137}\) and influenced its functions.

Results: Treatment of the wild-type R-spondin1-overexpressing HT1080 cells with tunicamycin, an inhibitor of N-glycosylation, resulted in a significant reduction in the molecular weight of R-spondin1. However, there was no effect in N-glycosylation-defective R-spondin1 mutant (N137Q)-overexpressing cells. These results demonstrated that R-spondin1 is N-glycosylated at only Asn\(^{137}\). We then evaluated the effects of N-glycosylation on its functions. R-spondin1 is a secreted protein and has Wnt signaling enhancing activity. We conducted comparative experiments with wild-type and N137Q mutant R-spondin1-overexpressing cells, and found that the secreted level of R-spondin1 was increased in N137Q mutant-expressing cells compared with wild-type cells. Furthermore, the Wnt signaling enhancing activity in N137Q mutant-expressing cells was higher than that of wild-type-expressing cells. These results suggested that N-glycosylation of R-spondin1 influences its secretion and Wnt signaling enhancing effect of R-spondin1.

Discussion: Our results show that R-spondin1 is N-glycosylated at Asn\(^{137}\) and suggest that R-spondin1 functions are regulated by N-glycosylation. We developed a detailed understanding of R-spondin1 to utilize as therapeutic adjuvants for eradicating metastatic cancers.

References
B67  

*N*-linked glycan which attached Asn\textsuperscript{354} regulate secretion of Extracellular matrix protein 1

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Introduction: Extracellular matrix protein 1 (ECM1) is a secretory protein that promotes angiogenesis and inhibits matrix metalloproteinase 9 (MMP9) activity. Overexpression of ECM1 is found in many epithelial tumors. Previously reported ECM1 as a marker for poor prognosis in breast cancer. In addition ECM1 gene mutations cause Lipoid proteinosis (LP) characterized by hoarseness and thickening of skin. Although ECM1 is proposed to be a secretory glycoprotein, the role of *N*-glycosylation remains unclear. The *N*-linked glycans attach to Asn residues at the consensus amino acid sequence, Asn-Xaa-Ser/Thr (where Xaa can be any amino acid except Pro) in the protein. Here, we determine the sites where *N*-linked glycan attached and elucidate the roles of *N*-glycosylation on ECM1.

Results and Discussion: To determine the *N*-glycosylated site in ECM1, we established ECM1-overexpressing HT1080 cells and purified recombinant ECM1 from conditioned media of the cells. Subsequent mass spectrometry analysis showed that ECM1 was *N*-glycosylated at Asn\textsuperscript{354} and Asn\textsuperscript{444}. To identify the function of *N*-glycosylation on ECM1, we established cell lines overexpressing mutant forms of ECM1 which substitute Asn (N) residue to Gln (Q) residue (N354Q, N444Q and 2NQ). We found that the level of secreted N354Q and 2NQ mutants, but not N444Q mutant, were higher than that of wild-type ECM1. These results indicate that *N*-linked glycan which attached Asn\textsuperscript{354} regulates secretion of ECM1. Moreover, we examined the effect of ECM1 mutations that are observed in LP patients (Q276X and W359X) on its secretion. We showed that the levels of secreted each mutant were lower than that of wild-type ECM1, contrary to N354Q mutants that participate in *N*-glycosylation, suggesting that *N*-linked glycan of ECM1 is not involved in LP.

Conclusion: It is indicated that *N*-glycosylation regulates secretion of ECM1 but it is not related to lipid proteinosis. Therefore it suggests that aberrance of *N*-glycosylation on ECM1 may promote angiogenesis. Moreover, these data could support the investigation for cancer metastasis.
B68 Colon cancer cells colonize the lung from established liver metastases through p38 MAPK signaling and PTHLH

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The mechanisms that allow colon cancer cells to form liver and lung metastases and whether KRAS mutation influences where and when metastasis occur is unknown. We provide clinical and molecular evidence showing that reduced p38 MAPK signaling endows cancer cells with the ability to form lung metastasis from previously established liver lesions. We found that reduced expression levels of MAP2K6, one of the major p38 MAPK activators, are associated with recurrence in CRC patients. In addition, downregulation of p38 MAPK signaling in two different colorectal cancer cell lines results in increased number of lung metastatic lesions, while liver metastatic growth is not affected. Colorectal cancer cells, in which p38 MAPK signaling was downregulated either by means of shRNAs or by use of inhibitor, show increased expression levels of PTHLH. Previous studies have implicated PTHLH in breast and squamous cell carcinoma tumor progression to bone metastasis through its activity in the bone remodeling process. Our work illustrates the systemic contribution of PTHLH released from tumor cells in supporting lung metastatic processes. We show that PTHLH can trigger Ca2+ release and AIFM1 mobilization leading to caspase-independent endothelial cell death, which, in turn destabilizes the vasculature by increasing permeability at the lung metastatic site. The release of PTHLH either from established liver lesions or from metastatic cells trapped at the lung vasculature is likely to increase lung endothelial permeability and facilitate metastatic cells extravasation.
How stromal cells assist tumor cells modulating the response to BRAF inhibition; impact for treatment of site-specific metastasis

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Tumor cells have the ability to recruit stromal cells forming a “metastatic niche” – growth permissive and protective microenvironment, which can weaken drug effects at metastatic sites [1]. It has been reported that the stroma-mediated protection phenomenon is particularly pronounced for targeted agents, like those targeting HER2 or mutated BRAF [2]. The aim of this study was to evaluate how different stromal cells modulate the response to the BRAF$^{V600E}$ inhibitor vemurafenib in metastatic melanoma. Melanoma cells were co-cultured with lung fibroblasts, endothelial cells or monocytes in vitro to mimic interactions with lung, vascular or inflammatory stroma during the treatment. We showed that the lung fibroblasts and endothelial cells, but not the monocytes reduced significantly melanoma sensitivity to the drug. By using intracellular flow cytometry we evaluated the status of different survival-signaling pathways. We showed the protective stroma-dependent elevation of pS6, which indicates the mTOR pathway activation in the treated melanoma cells. When the mTOR inhibitor everolimus was added, the protective effect of the stroma was eliminated and the melanoma sensitivity to vemurafenib was restored. This indicates that mTOR activation might be one of the mechanisms how the protective-stroma enables innate “resistance” to vemurafenib. Currently, we are investigating how organ-specific stroma (from different metastatic sites) affects drug-response in established metastases in vivo. For this, we generate experimental metastases in multiple organs followed by systemic treatment with vemurafenib. Subsequently, the metastatic cells are analyzed for the status of mTOR (and other signaling pathways) aiming to reveal whether there are site-specific differences in response. The knowledge regarding stromal influence on the drug-response might be useful searching for indicative biomarkers or designing new drug combinations targeting site-specific metastasis.

Elucidating the mechanisms of IL4Rα-induced mammary tumor growth

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Interleukin-4 (IL4), a Th2 cytokine, and the IL4/IL4 receptor (IL4R) interaction have well defined roles in promoting the proliferation and survival of activated lymphocytes. This is controlled by several signaling pathways including the PI3K/Akt pathway, and is supported in part by IL4-induced glucose metabolism. Interestingly, IL4Rs are overexpressed in many epithelial cancers including breast cancer, yet whether they drive the proliferation and survival of breast cancer cells is unknown. To determine the functional significance of IL4R expression, we used RNA interference to knock down (KD) IL4Rα, a signaling component of the IL4R, in two independent murine mammary cancer cell lines (R221a and 4T1). Using control or IL4Rα KD clones in orthotopic or experimental metastasis models, we found that IL4Rα expression is a strong promoter of both primary (mammary gland) and metastatic (lung and liver) mammary tumor growth. Using small molecule inhibitors in colony formation assays, we determined that pErk1/2, pAkt, and pmTor each mediate enhanced colonization ability in response to IL4 in vitro. Immunohistochemical analysis of R221a and 4T1 lung metastases also showed significantly reduced levels of pAkt (s473) and pErk1/2 in IL4Rα KD tumors compared to controls, indicating a role for Akt and Erk in mediating the pro-tumor effects of IL4Rα in vivo. In agreement with the concept that IL4-induced glucose metabolism could support mammary tumor growth, we found that IL4-induced cell number correlates with an increase in glucose consumption and lactate production in 4T1 cells. In addition, IL4-induced protein expression of GLUT1, the main glucose transporter upregulated in epithelial cancers, was attenuated in IL4Rα KD cells compared to controls in vitro. In vivo, GLUT1 levels examined by immunohistochemistry were significantly reduced in orthotopic 4T1 IL4Rα KD tumors compared to control tumors. Finally, pharmacologic inhibition of hexokinase-2, the enzyme controlling the initial rate-limiting step in glycolysis, blocked IL4-induced cell number, implicating a causal relationship between IL4-induced glucose metabolism and increased growth. Collectively, our results demonstrate that the IL4 receptor promotes the growth of mammary cancer cells at primary and metastatic sites, possibly through activated Akt, Erk and mTor signaling, in addition to the induction of glucose metabolism. Drugs targeting IL4Rα signaling may have potential for treating primary breast cancers and limiting metastatic disease.
**B71  miR-34a Blocks Osteoporosis and Cancer Bone Metastasis by Inhibiting *Osteoclastogenesis and Tgif2**

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The bone resoring osteoclasts significantly contribute to osteoporosis and cancer bone metastases. MicroRNAs (miRNAs) play important roles in physiology and disease, and present tremendous therapeutic potential. Nonetheless, how miRNAs regulate skeletal biology and bone metastases is underexplored. Here we identify miR-34a as a novel and critical suppressor of osteoclastogenesis, bone resorption and the bone metastatic niche. miR-34a is down-regulated during osteoclast differentiation. Osteoclastic miR-34a over-expressing transgenic mice exhibit lower bone resorption and higher bone mass. Conversely, miR-34a knockout and heterozygous mice exhibit elevated bone resorption and reduced bone mass. Consequently, ovariectomy-induced osteoporosis, as well as breast and skin cancers, are diminished in osteoclastic miR-34a transgenic mice, and can be effectively attenuated by miR-34a nanoparticle treatment. Mechanistically, we identify Tgif2 (transforming growth factor-beta-induced factor 2) as an essential direct miR-34a target that is pro-osteoclastogenic. Tgif2 deletion reduces bone resorption and abolishes miR-34a regulation. Together, using mouse genetic, pharmacological and disease models, we reveal miR-34a as a key osteoclast suppressor and a potential therapeutic strategy to confer skeletal protection and ameliorate bone metastasis of cancers.
Spliced Osteopontin Regulates the Energy Metabolism during Anchorage-Independence

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The major limiting factor in the process of metastasis formation is the death of the tumor cells before their implantation in the target organs. Hence, anchorage-independent survival is essential for metastasis. While cancer cells can survive in the circulation for extended periods of time, untransformed non-hematopoietic cells undergo anoikis consecutive to losing contact with their substratum. The detachment of mammary epithelial cells prompts a loss of glucose transport and ATP deficiency, thus compromising the energy metabolism. Invasive breast tumor cells abundantly express two splice variants of the metastasis gene osteopontin. Osteopontin-a and osteopontin-c synergize in supporting tumor progression via up-regulating the energy production, leading to deadherent survival.

- The full-length form, osteopontin-a, induces a gene expression profile that is associated with tissue remodeling and directed movement/sprouting. Osteopontin-a increases the levels of glucose in breast cancer cells, likely through sn-glycero-3-phosphocholine, STAT3 and its transcriptional targets apolipoprotein D and IGFBP5.

- Osteopontin-c signaling activates three interdependent pathways of the energy metabolism. It supports the hexose monophosphate shunt and glycolysis that can feed into the tricarboxylic acid cycle, leading to mitochondrial ATP production. Up-regulation of the glycerol phosphate shuttle also supports the mitochondrial respiratory chain. Drawing substrates from glutamine and glycolysis, elevated creatine may be synthesized from serine via glycine and supports the energy metabolism by increasing the formation of ATP. Metabolic probing identified differential regulation of the pathway components, with mitochondrial activity being redox dependent and the creatine pathway depending on glutamine. The osteopontin-c induced pathways generate a flow toward two mechanisms of ATP generation, via creatine and the respiratory chain. These mechanisms are consistent with a stimulation of the energy metabolism that supports anti-anoikis. Osteopontin-c is never expressed without the full-length form osteopontin-a. Each of these forms activates signal transduction pathways that are distinct from the other. Our findings imply a coalescence in cancer cells between osteopontin-a, which increases the cellular glucose levels, and osteopontin-c, which utilizes this glucose to generate energy. The splice variant-specific metabolic effects of osteopontin add a novel aspect to the pro-metastatic functions of this molecule. It is likely that metabolic responses to environmental cues are more common in cell biology than has hitherto been recognized.
B73  The Hippo Pathway Controls Cancer Cell Vascular Invasion and Metastasis by Modulating Cytokine Signalling

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Cancer cell metastasis requires local vascular invasion and extravasation at a distant organ site. Here, we show that the vascular invasiveness of a series of commonly studied cancer cell lines can be altered by the density at which the cells are propagated. Invasion through an endothelial monolayer in vitro was inhibited among cells grown at high density and enhanced among cells grown at low density. This reversible phenotype was also observed during vascular invasion and extravasation of cancer cells into stromal tissues in a zebrafish model. A kinome-targeted RNAi screen for the drivers of vascular invasion showed enrichment of shRNAs targeting the LATS1 kinase that phosphorylates and inhibits the function of YAP in the Hippo pathway. YAP depletion or inhibition by verteporfin reduced the invasiveness of cancer cells in vitro and in vivo as well as metastasis. The invasive phenotype was associated with the YAP-dependent up-regulation of immune response genes that included the cytokines IL6, IL8, and CXCL1, 2, and 3. Blockade of the cytokine receptors confirmed that they promote cancer cell vascular invasiveness.
B74 RAD51 supports metastasis via three major mechanisms independent of DNA repair.

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Recently, the seminal discovery that metastatic breast cancer cells up-regulate DNA damage repair proteins in order to stabilize an unstable genome has sparked substantial interest in deciphering the mechanisms supporting cancer progression and targeting these molecules therapeutically. We have identified a key homologous recombination (HR) DNA repair protein, RAD51 that is overexpressed in patient breast metastases samples compared to matched primary breast cancer. We find depletion of RAD51 inhibited metastases in a murine, syngeneic breast cancer model and xenograft transplants. Corresponding with inhibition of metastasis we observed changes in cell morphology, pro-metastatic gene expression profile and immune signaling. We believe RAD51 is functioning as a transcriptional co-factor influencing three related mechanisms via changes in expression of; Rho and Rac GTPases affecting actin dynamics, matrix-metallo-proteases affecting interaction with the surrounding environment, and chemokine receptor CXCR4 affecting cell signaling. The combination of these influences results in effective inhibition of metastasis at multiple functional levels. This demonstrates for the first time a new activity for RAD51 that may underlie the proclivity of patients with RAD51 overexpression to develop distant metastasis. Therefore we suggest RAD51 is a potential biomarker and attractive drug target for metastatic cancers.
B75 Implication of endothelial Notch signaling on tumor metastasis

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While more and more insights are gained in the processes of tumor cell invasion and access to the circulation, less is known about later steps during the metastatic cascade. The Notch pathway, a highly conserved cell signaling mechanism for intercellular communication, is important for many cell fate decisions during vascular development. Here, we show that vascular Notch signaling affects interactions between tumor cells and endothelial cells.

The activation of Notch signaling in endothelial cells led to increased adhesion and transmigration of different tumor cell lines in vitro. This was accompanied by increased expression of endothelial adhesion molecules, which are usually known to promote the extravasation of leukocytes. Consistently, active Notch signaling in endothelial cells caused also stronger interactions with tumor cells in a flow chamber and slowed down the speed of rolling tumor cells on an endothelial monolayer. We could show that NOTCH1 mediated effects on adhesion are transcription dependent. Interestingly, the induction of adhesion molecule expression by Notch signaling was independent of NFκB signaling. Furthermore, Notch activation changed the chemokine profile of the endothelium and promoted tumor cell transmigration towards endothelial monolayers. In agreement with our in vitro studies vascular-specific Notch signaling activation increased experimental metastasis to the lung as well as endogenous metastases formation of an experimental primary tumor in syngeneic mouse tumor models.

Identification of underlying mechanisms and functional characterization of responsible downstream targets of the Notch pathway are ongoing. This may provide novel insights in the mechanisms of tumor cell adhesion and extravasation at the vessel wall of secondary tumor sites.
High content multiparametric functional screen for regulators of epithelial-mesenchymal transition identifies genes associated with chemoresistance

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The phenotypic transition of non-motile epithelial tumour cells to migratory and invasive ‘mesenchymal’ cells provides a mechanism that enables the escape of cancer cells from the primary tumor to distant sites. Morphological changes from cobblestone epithelial cells to elongated and spindle-shaped cells is a key feature of this process. This transition is also marked by upregulation of vimentin, an intermediate filament found in mesenchymal cells. We have previously developed a bladder cancer model with a step-wise enhancement in the metastatic cascade that we have used to interrogate the role of tumor cell plasticity in metastasis (1). Given the need to elucidate pathways and assign functions to particular genes involved in this process, we conducted a high content screening assay across genome wide siRNA, miRNA and kinase inhibitor (131 compounds) libraries to systematically identify modulators of tumour cell plasticity in the context of epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET). We identified candidate gene regulators of EMT in TSU-Pr1-B1 bladder cancer cells using the readouts of morphological change (cells stained with CMFDA) and a vimentin promoter activity (dsRed reporter construct). Following integration of the predicted target genes for miRNA hits and kinase inhibitors with siRNA hits, initial validation using a siRNA deconvolution strategy was performed for 400 targets. 221 siRNAs were classified as inducing statistically significant effects on cell shape and/or vimentin promoter reporter activity (defined as at least 2/4 duplexes inducing the effect for a given target). Gene ontology and pathway analyses feature cell cycle and various developmental pathways. Furthermore, the validated gene list was associated with bladder cancer histology and drug sensitivity in clinical specimens in multiple cancer types (Oncomine). Molecules identified in this assay will be further analysed using functional assays to determine their role in maintaining the epithelial phenotype and as regulators of chemoresistance.

B77 Breast cancer cells modulate the hyaluronan-matrix of mesenchymal stem cells, thereby forming a pro-metastatic environment in vitro.

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Homing and survival of cancer cells in the metastatic niche depends on a supporting microenvironment. Mesenchymal stem cells (MSC) are multipotent progenitor cells in the bone marrow and potent modulators of the microenvironment. Hyaluronan (HA) as a chief component of the extracellular matrix promotes cancer progression and increases the metastatic potential of cancer cells. Aim of the present study is therefore to investigate the modulation of the MSC HA-matrix by tumor cells and its effect on metastasis in vitro.

Mono-cultures of MSC were stimulated with conditioned supernatant of three different breast cancer cell lines for 48h (MCF-7, TMX2-28, MDA MB 231). Changes of the HA-matrix were analysed by qRT-PCR, immunocytochemistry and ELISA. Furthermore the migratory potential of breast cancer cells (BCC) was investigated by performing transwell migration assays, scratch assays and cell tracking via time lapse microscopy.

HA-synthases 1-3 (HAS1-3) and the HA-degrading enzymes, Hyaluronidases (Hyal), were analysed in MSC by qRT-PCR. HAS2 was found to be the prevalent HA-synthase (96%, HAS3 3%, HAS1 1%) in MSC, whereas Hyal2 is the foremost expressed hyaluronidase. MSC were stimulated with the conditioned supernatant of BCC. After 24 hours the concentration of HA in the “double-conditioned” medium was analysed by HA-ELSA. HA was found to be increased after treatment with BCC supernatant compared to control levels. This effect could be abolished by simultaneous treatment with the HAS-inhibitor 4-MU. In addition, indirect co-culture experiments were performed to assess consequences of an altered MSC HA-matrix on the BCC invasive phenotype. Pre-conditioned MSC attracted BCC in a HA dependent manner.

Further analysis of HAS and Hyal mRNA levels led to the hypothesis that MCF-7 cells induce the emergence of small HA-fragments (sHA) whereas TMX-2 and MDA-MB cells lead to the synthesis of high molecular weight HA (HMW-HA). sHA and HMW-HA have previously been reported to possess different biological functions. Therefore, BCC were treated with sHA and HMW-HA and cell motility was analysed by time lapse microscopy. Whereas MCF-7 responded solely to sHA (1.46±0.18 fold of control) TMX2 and MDA showed an increased motility when treated with HMW-HA (TMX: 1.33±0.12; MDA: 1.5±0.16; fold of control).

In conclusion, BCC appear to alter the MCS HA-matrix, thereby possibly creating a pro-metastatic microenvironment.
A regulatory motif analysis implicates NF-κB1 and HIF1α transcription factors as molecular mediators of breast cancer metastasis to the brain

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Clinically relevant metastases in the brain are diagnosed in 25%-30% of patients with disseminated breast cancer. The poor prognosis, 80% mortality within one year and short median survival time mirror the lack of targeted therapies. The absence of robust preclinical models which mimic the entire metastatic cascade including the dissemination from the primary tumor to the colonization of the brain, limits research on cellular and molecular mediators of brain metastasis.

Based on the murine 4T1 breast cancer cell line, a unique syngeneic and orthotopic model of spontaneous brain metastasis (4T1-BM²) with 100% penetrance in immunocompetent Balb/c mice, was recently developed in our group. Transcriptome analysis coupled with breast cancer patient datasets mining identified several molecular targets with potential clinical relevance in brain metastatic progression. In parallel, an Integrated System for Motif Activity Response Analysis (ISMARA) was performed to computationally predict activity of regulatory sites for Transcription Factors (TFs) and micro-RNAs (miRNAs). Interestingly, Hypoxia-inducible factor 1-alpha (HIF1α) and Nuclear Factor NF-kappa-B p105 (NF-κB1) transcription factors were predicted to be highly active in 4T1-BM² compared to the non-brain metastatic control cell lines, 4T1-T². Using a Luciferase reporter assay we confirmed elevated activity of both HIF1α and NF-κB1 in 4T1-BM² cells. Preliminary data suggest a sequential activation of the two transcriptional regulators with a feedback loop involving let-7 miRNA in brain metastatic breast cancer cells. Silencing of HIF1α and NF-κB1 using shRNA approaches will provide further evidence for the involvement of those pathways in the formation of brain metastasis.

A potential role for the transcriptional activity of both TFs in controlling the expression of several brain metastasis-promoting molecular targets is currently being investigated.
Early tumor shrinkage as a potent predictor of outcome in KRAS wild-type colorectal liver-limited metastases treated with cetuximab plus chemotherapy: lessons from a randomized, controlled trial

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Background:
Recently, early tumor shrinkage (ETS) was reported to predict outcome in metastatic colorectal cancer treated with cetuximab (cet). This study was to evaluate the impact of ETS on long-term outcome in patients (pts) with wild-type-KRAS unresectable LLM receiving cet plus chemotherapy (CT, FOLFIRI or mFOLFOX6).

Material and Methods:
138 pts treated in a randomized controlled trial (70 in armA received CT plus cet and 68 in armB received CT alone) previously reported (Jianmin et al, ESMO 2012, abstract-557, ClinicalTrials.gov, number NCT01564810) were included into this analysis. ETS was defined as a reduction of ≥20% in the sum of the longest diameters of target lesions compared to baseline at the first evaluation (8 weeks). Outcome measures were progression-free survival (PFS) and overall survival (OS).

Results:
132 pts were available for evaluation, and ETS occurred more frequently in armA than that in armB (45/68 vs. 26/64, p=.003). Irrespective of treatment arm, pts achieved ETS were associated with longer OS (armA: 38.0 vs. 18.7months, p<.001; armB 30.6 vs. 17.7months, p=.003) and PFS (armA: 11.8 vs. 4.8months, p<.001; armB 8.0 vs. 4.6months, p=.001) when compared to pts with no-ETS. Among pts with ETS, there were statistic difference between armA and armB in terms of PFS (11.8 vs. 8.0months, p=.041) but not of OS (38.0 vs. 30.6months, p=.30); the converted resection rates for liver metastases were 40.0% (18/45) in armA and 19.2% (5/26) in armB, which were no significantly different (p=.072). For pts without liver surgery, pts observed ETS also gained an increased survival benefit over those no-ETS in armA with regards to OS (p=.01) and PFS (p<.001) though it was not full certified in armB (OS: p=.054; PFS: p=.041). For pts in armA, cet-induced skin toxicity correlated with the occurrence of ETS (p=.048). In addition, cox regression for OS using indicated a hazard ratio of 0.39 (95%CI 0.21–0.72, p=.003).

Conclusions:
ETS ≥20% at 8 weeks may serve as a predictor of favorable outcome in pts with wild-type-KRAS CLLM receiving cet plus CT.
Molecular characterization of early disseminating cancer cells in Non-Small Cell Lung Cancer

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Non-small cell lung cancer (NSCLC) is the most common cause of death. Despite diagnostic progress, NSCLC is often diagnosed at an advanced stage. Moreover, even patients diagnosed with disease stages I and II that are treated with surgery develop frequently wide spread metastasis within 5 years, indicating that NSCLCs disseminate early. In order to uncover the genomic diversity and evolution of early disseminated cancer cells (DCC) we aimed to isolate the cells and perform array Comparative Genome Hybridization (aCGH). Lymph Node (LN) and/or Bone Marrow (BM) samples were obtained during surgery. DCCs were identified using epithelial markers (EpCAM for LN-DCCs and cytokeratin (CK) for BM-DCC) and picked using a micromanipulator. Thereafter, their genomic DNA was amplified globally. Microdissected primary tumor (PT) and metastatic lesions (Met) of patients were also processed. Samples with high quality DNA were selected for aCGH analysis.

Amongst the 148 NSCLC patients DCCs were detected in 86 cases in LN and/or BM samples (58%). Six cases with various histologies (Adenocarcinoma (ADC), squamous cell carcinoma, ADC in situ) at M0 or M1 stages were analyzed using aCGH. From these patients we had isolated 22 DCCs and 7 control cells (EpCAM- or CK-). All of the LN-DCCs, PTs, Mets from adrenal gland and cerebellum, as well as 6/8 BM-DCCs showed mostly heterogeneous aberrations; whereas none of the normal cells displayed aberrations. BM-DCCs harbored fewer aberrations compared to LN-DCCs. PT samples displayed fewer alterations than Met samples but more than DCCs.

In conclusion, we observed an unexpected degree of heterogeneity among local and systemic cancer. The findings suggest different lines of molecular evolution in NSCLC, which require detailed analysis of DCCs to understand progression towards metastasis.
B81 Nuclear localization of Kaiso is a prognostic biomarker in African American Women and promoter of EMT in infiltrating ductal breast carcinomas.


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Background: The expression and biological consequences of Kaiso, a novel BTB-POZ bi-modal transcription factor, in infiltrating ductal carcinomas (IDCs) have not been widely investigated.

Methods: Kaiso expression and subcellular localization was determined in 146 normal tissues, 376 IDCs, and 85 lymph node metastases by immunohistochemistry. Semi-quantitative analysis of staining was evaluated with clinicopathological features of IDCs using Chi-square analysis and Kaplan Meier Curves. MCF-7 cells were induced to overexpression Kaiso, whereas MDA-MB-468 and MDA-MB-231 cells were treated with si-Kaiso or si-Scr and assayed for cell migration, invasion, RNA and protein expression. Chromatin Immunoprecipitation assay was performed in MDA-MB-231 cells to determine the direct interaction of Kaiso with methylated sequences within E-cadherin promoter. Results: Kaiso expression in both the cytoplasmic and nuclear compartments correlated with age <48 (cytoplasmic p<0.0093; nuclear p< 0.0001) and moderate differentiation (cytoplasmic p<0.0042; nuclear p<0.0001). However, only nuclear Kaiso correlated with poor prognostic factors, i.e., race (African Americans) (p<0.0001), poor differentiation (p<0.0001), and metastases (p<0.0001). Nuclear Kaiso was also associated with worse overall survival (p<0.0019), with African American patients displaying worse survival rates relative to Caucasian patients (p<0.029). Over-expression of Kaiso in MCF-7 cells increased cell migration and invasion, but treatment of MDA-MB-468 and MDA-MB-231 cells with si-Kaiso decreased cell migration and invasion. Treatment also induced the expression of E-cadherin and a reversal of mesenchymal associated cadherins, N-cadherin and cadherin 11, as well as decreased vitmenin expression. Further, Kaiso directly bound to methylated sequences in the E-cadherin promoter, an effect prevented by 5-aza-2-deoxycytidine. Lastly, immunofluorescence co-staining of poorly differentiated IDCs demonstrated that nuclear Kaiso is associated with a loss of E-cadherin expression.

Conclusions: Collectively, these findings support an oncogenic role for Kaiso in promoting aggressive breast tumors.
B82 MiR-212 suppresses the metastasis of lung cancer cells via targeting Metalloproteinase 16 (MMP16)

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MicroRNAs (miRNAs) are small, non-coding RNAs that play crucial roles in the development of human cancer. The contributions of miRNAs in the metastasis of human lung cancer still need to be further elucidated. In the present study, we performed microRNA chip assay for the first time to screen the differentially expressed miRNAs between the highly and lowly metastatic non–small cell lung cancer (NSCLC) cells. Selectively, miR-212 was of interest in our study. We demonstrated that the levels of miR-212 were dramatically decreased in examined NSCLC cell lines and clinical tissues. Moreover, we found that histone H3- K27 methylation in the promoter of miR-212 is responsible for the down-regulation of this microRNA. Next, we demonstrated that introduction of miR-212 greatly suppressed the migration, invasion, and metastasis of lung cancer cells in vitro and in vivo, suggesting that miR-212 may be a novel tumor suppressor. Further investigations revealed that metalloproteinase 16 (MMP16) was one of direct target genes of miR-212 which reduced the expression of MMP16 at the levels of mRNA and protein. Moreover, knockdown of MMP16 was able to inhibit the migration and invasion of lung cancer cells, functionally resembling to the effect of miR-212 overexpression. Meanwhile, silencing MMP16 expression reversed the enhanced migration and invasion mediated by anti-miR-212. These results suggest that miR-212 suppresses metastasis of lung cancer cells through directly targeting MMP16. Thus, our finding provides new insight into the mechanism of NSCLC metastasis, indicating a therapeutic potential of miR-212 in the treatment of human NSCLC.
B83 Targeting Driver Kinases as Novel Therapies for Breast Cancer Brain Metastases

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Of 1.6 million newly diagnosed breast cancer patients per year, about 10-16% will develop brain metastases. Among the different subtypes of breast cancer, HER2-overexpressing (HER2+) and triple-negative breast cancers (TNBC) have the highest incidence of brain metastasis. Recently, advances in targeted therapies for breast cancer (e.g., trastuzumab, T-DM1, and lapatinib) have prolonged patient survival through better control of the systemic disease; however, when patients have disease recurrence, their brain metastasis incidence doubles, which represents an emerging challenge. Current treatment options are limited and merely palliative for those patients with brain-metastatic breast cancer, and their one year survival is less than 20%. To develop effective treatments for brain metastasis, we strived to identify druggable targets (driver kinases) that promote breast cancer brain metastasis to guide rapid development of novel and clinically applicable targeted therapies for breast cancer brain metastasis. Many kinases activate cell signaling pathways critical in cancer development. In the last decade, kinase inhibitors are among the most successful targeted cancer therapies and their clinical efficacy has ushered in the era of personalized medicine. However, all existing inhibitors have been developed based on knowledge of primary tumors; no efforts have yet been reported to identify metastasis-specific, druggable molecular targets. Here we aim to identify kinases nonessential for primary tumor growth but may be critical for colonization of the brain and outgrowth of metastases, thereby imbuing metastatic cancer cells with brain-specific growth advantages and serving as ideal therapeutic targets. Therefore, we have performed an unbiased in vivo kinome screen in brain metastasis animal model to uncover novel targets for effective brain metastasis therapy. We divided an activated kinase open reading frame (ORF) library into 17 pools (artificial heterogeneity) and introduced the kinase ORF library into the MDA-MB-231 breast cancer cell line that has been labeled with GFP and Luciferase. We injected these kinase-harboring cells by intracarotid injection into nude mice to identify kinases that promote brain metastasis by in vivo imaging. Several pools led to dramatically reduced mouse survival due to brain metastasis compared to vector control cells. A pilot next-generation sequencing (NGS) experiment identified that these aggressive brain metastases were indeed enriched with several oncogenic kinases in vivo. Among these were three kinases, SPHK1, FRK, and MAPK12, previously unreported in brain metastasis but with available inhibitors that are either FDA-approved or are tested in clinical trials for treating other diseases. We have validated a top candidate in breast cancer brain metastasis in vivo, demonstrated its clinical relevance in patients brain metastasis samples, and used an available kinase inhibitor to target the top kinase in brain metastasis model in vivo. We are excited the kinase inhibitor doubled life span in 60% of mice bearing breast cancer brain metastases. Currently, are completing NGS of the remaining samples, functionally validating other top candidates, and exploring the potential of using the available kinase inhibitors to target these kinases for the treatment and/or prevention of breast cancer brain metastasis in vivo. Targets identified and validated in this manner may serve as the first generation of breast cancer brain metastasis-targeted therapies for fast-track clinical application to help those patients in dire need.
Characterization of a new rat monoclonal antibody against carbohydrate deficient bone sialoprotein in terms of binding and anti-metastatic properties.

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Osteolytic skeletal metastasis is a leading event during breast cancer progression. At this stage of the disease, there is no curative treatment available, although it is possible to slow down the progression of the disease or to reduce the complications resulting from affected skeletal areas, such as pain and instability. For this reason, a therapy especially directed against bone metastases is highly desirable. Besides others, bone sialoprotein II (BSPII), which is found in extracellular bone matrix, has attracted interest as a marker of skeletal lesions. Elevated concentrations of the secreted SIBLING protein have been detected in the serum of breast cancer patients and correlate with poor prognosis. Based on these facts, it was hypothesized that BSPII could serve as valuable target for anti-metastatic therapy.

This protein is recognized by a rat monoclonal antibody (Anti-CD-BSP mab), which binds to a hypo-glycosylated threonine in position 109 of the amino acid chain. In an attempt to characterize the therapeutic potential of this antibody, a cell panel (human breast cancer cells MDA-MB-231 (metastatic), human prostate cancer cells PC-3 (metastatic), human non-metastatic breast cancer cells MCF-7, and human immortalized breast cells MCF-10) was used to evaluate its binding properties. In addition, the anti-proliferative, anti-migratory and anti-metastatic properties of this antibody were determined in MDA-MB-231 cells by proliferation and migration assays, as well as by an in vivo experiment. To that purpose, \(1 \times 10^5\) MDA-MB-231 cells were inoculated into the femoral artery of male nude rats and the appearance of osteolytic lesions in the respective hind leg was monitored by photon emission, mediated by the metabolism of luciferin. In the treatment arm of this study, the antibody was administered subcutaneously at a dose of 10 mg/kg/week over six weeks and treatment started when tumor bearing rats had shown stable tumor growth. The antibody against carbohydrate deficient (CD) BSP showed primarily affinity for large cells of the metastatic cell lines. Small metastatic cells did not express CD-BSP, but medium-sized cells showed various patterns of both CD-BSP and normal BSP expression. In large cells, the CD-BSP expression was always accompanied by that of normal BSP, mainly distributed on the outer cell membrane. In special cases, CD-BSP was present in the cell nucleus, as verified by 3D-confocal laser scanning microscopy. The non-metastatic cells did not express CD-BSP.

In MDA-MB-231 cells, there was no anti-proliferative or anti-migratory activity following exposure to this antibody (up to 900\(\mu\)g/ml). However, in nude rats implanted with MDA-MB-231 cancer cells, this antibody showed a remarkable activity against lytic skeletal metastasis, as shown by complete remissions in more than 70% of the rats and a no change situation in the remaining part. In contrast to this result, 96% of rats of the control arm showed rapid tumor growth accompanied with lytic destruction of femur and tibia of the respective hind leg. In conclusion, the monoclonal rat antibody directed against carbohydrate deficient BSPII is a powerful tool for treating experimental skeletal metastasis and warrants further development.
Elevated HOXB9 expression promotes differentiation and predicts a favorable outcome in colon adenocarcinoma patients

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BACKGROUND: Little is known about the tumor suppressive proteins and the underlying mechanisms that suppress colon cancer progression. Homeodomain-containing transcription factor HOXB9 plays an important role in embryogenesis and cancer development. We here aim to uncover the potential role of HOXB9 in the regulation of epithelial-to-mesenchymal transition in colon adenocarcinoma progression.

METHODS: HOXB9 expression in colon adenocarcinoma cells and patients was analyzed by Western blot and immunohistochemistry separately. Correlation between HOXB9 expressions with patients’ survival was assessed by Kaplan-Meier analysis. HOXB9 regulated target gene expression was determined by RNA-sequencing in HOXB9 overexpressing colon adenocarcinoma cells.

RESULTS: Elevated HOXB9 expression was identified in well-differentiated colon adenocarcinoma patients and was associated with better overall patients’ survival. Overexpression of HOXB9 inhibited colon adenocarcinoma cell growth, migration, invasion in vitro and tumor growth, liver as well as lung metastases in nude mice; whereas silencing HOXB9 promoted these functions. HOXB9 promoted colon adenocarcinoma differentiation via a mechanism that stimulates mesenchymal to epithelial transition, involving downregulation of Snail, Twist, FOXC2 and ZEB1 and upregulation of E-cadherin, Claudins 1, 4, 7, Occludin and ZO-1.

CONCLUSION: HOXB9 is a novel suppressor that inhibits colon adenocarcinoma progression by inducing differentiation. Elevated expression of HOXB9 predicts longer survival in colon adenocarcinoma patients.
Elevated Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3) is a poor prognostic marker in pancreatic ductal adenocarcinoma (PDAC)

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Aims. Pancreatic cancer, characterized by a high mortality, rapid progression, and resistance to chemo and radiation therapy, is one of the most fatal gastrointestinal (GI) cancers, with only around 5% survival rate beyond five years after initial diagnosis. Inflammation has led to a growing area of interest in pancreatic cancer progression, in particular the creation of a favorable tumorigenic conditions and aggressively resistant metastasis. Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3) gene from IFITs family is among hundreds of IFN-stimulated genes. The potential role of IFIT3 in cancer is scarcely understood. We have found that overexpression of the gene IFIT3 enhances tumor growth, angiogenesis, metastasis and chemoresistance of the pancreas carcinoma cells. However, the clinical relevance of IFIT3 is not yet known in pancreatic cancer. We will analyze the prognostic significance of this gene in this tumor entity.

Methods. Expression of IFIT3 were detected in a panel of pancreatic ductal adenocarcinoma cell lines (AsPC-1, BXPC3, Capan-2, HPAF-2, MiaPaCa, SW1990), pancreatic cancer cell lines with different metastatic potential (FG and L3.6pl) and two established gemcitabine resistant cell variants. To evaluate the clinical relevance, we applied tissue microarray (TMA) analysis by performing an immunohistochemical assessment of IFIT3 in surgical samples from total 274 consecutive patients affected by pancreatic adenocarcinoma. The prognostic significance of IFIT3 staining intensity was evaluated.

Results. L3.6pl cells present an aggressive capacity of tumor growth, metastasis and angiogenesis as compared to FG cells, and showed a significant higher expression of the IFIT3 gene. Over-expression of the IFIT3 led to a significant increase in IL-6 production. In 274 PDAC tissues, 20.8% was IFIT3 negative expression while 46.4% case displayed weak expression and 32.9% of patients showed middle or high expression. The PDAC patients with high expression of IFIT3 statistically linked to higher staging and with shorter survival (p=0.042) Multivariate analysis showed that pathological stage and grading and IFIT3 over-expression were statistically associated with poor prognosis.

Conclusions
High expression of IFIT3 was independently correlated to shorter patients survival and could serve as a prognostic marker. Inhibition of IFIT3 might provide a new strategy for PDAC therapeutic and diagnostic procedures.
B87 Aberrant microRNAs expression in lung cancer stem cells with different metastatic potential lung cancer cells*

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Background It has been proven that CSCs plaid an important roles in cancer initiation, therapy resistance, recurrence, and metastasis, and has been identified in several solid tumors, including lung cancer. However, the regulation mechanism of CSCs in lung cancer is still uncertain, and some miRNAs have been functionally linked to CSCs. To get better understanding of how miRNAs drive or suppress the functional mechanisms behind the generation and maintenance of cells with stem-like properties, a subpopulation of CSCs was enriched from non-small cell lung cancer(NSCLC) cell lines with different metastatic potential, and the differential expression profiles of miRNAs between them were analyszed.

Methods High metastatic lung cancer cell lines L9981-Luc and low metastatic lung cancer cell line NL9980-Luc were plated in stem cell conditioned culture system allowed for sphere forming, which was followed by cisplatin selection. In order to evaluate the stemness characteristics of spheres, the self-renewal, proliferation, differentiation, chemoresistance, migration, invasion and tumorigenicity of the L9981-Luc and NL9980-Luc sphere-forming cells, as well as the expression levels of candidate stem cell markers were assessed, comparing with the parental cells. We next investigated aberrant miRNAs in the L9981-Luc sphere-forming cells compared to the NL9980-Luc sphere-forming cells with miRNA expression profile. Some changed miRNAs were validated by reverse transcription and real-time PCR (RT-PCR). Target genes of miRNAs were predicted using three major online miRNA target prediction algorithms: TargetScan, miRanda and miRWalk. KEGG analysis was applied to analyze the main function of the predicted target genes.

Results The L9981-Luc and NL9980-Luc could form clonal nonadherent spheres and be serially passaged. The L9981-Luc and NL9980-Luc sphere-forming cells displayed a greater self-renewal capacity as demonstrated by higher levels of sphere formation and stem cell marker(CD133, CD44) expression, contained a larger proportion of side population cells, showed increased drug-resistant properties and exhibited higher tumorigenicity in nude mice. In the presence of serum medium, the sphere-forming cells could adhere to the plastic and acquired the typical morphologic features of differentiated cells, and showed an lower expression of CK18 than the differentiated cells in the adherent condition. Although the L9981-Luc sphere-forming cells had a greater capacity of migration and invasion than L9981-Luc cells, there were no significant differences in migration and invasion between NL9980-Luc sphere-forming cells and NL9980-Luc cells. Interestingly, the L9981-Luc sphere-forming cells exhibited a higher ability of migration and invasion than NL9980-Luc sphere-forming cells, and generated larger xenograft tumors than NL9980-Luc sphere-forming cells. Then, 41 miRNAs with significant difference were identified between L9981-Luc and NL9980-Luc sphere-forming cells. Some aberrant miRNAs were chosen to perform quantitative RT-PCR. MiRNA-137, miRNA-499a, miRNA-215,
miRNA-339-5p and miRNA-378a-5p were down-regulated, while miR-30a-5p, miR-181c-5p, miRNA-221-5p and miRNA-222-3p were up-regulated in L9981-Luc sphere-forming cells when compared to NL9980-Luc sphere-forming cells. KEGG analysis suggest that target genes are related with MAPK signaling pathway, Insulin signaling pathway, p53 signaling pathway, TGF-beta signaling pathway, ErbB signaling pathway and Wnt signaling pathway.

**Conclusion** Non adherent tumor spheres from L9981-Luc and NL9980-Luc in stem cell conditioned medium containing DDP possess CSCs properties. And there might be distinct populations of CSCs in a specific tumor which is associated with different miRNA expression profiling.

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Studies on the Relationship Between the Natural Killer Cell Receptor Status and Tumor Microenvironment and Metastasis of Lung Cancer

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Background: Lung cancer is one of the most common malignant tumors in the world, which has high morbidity and mortality and accounting for about 25.4% of all tumors. It has been an upward trend of the incidence rate in recent years [1-4]. The American Cancer Society released data show that 222,520 cases of respiratory cancer and 157,300 cases of death in 2010, which is in the first place of morbidity and mortality of all malignant tumors [5]. A clinical statistics of stage IV NSCLC in China showed that the 1-,2-,3-,4- and 5-year survival rate was 44%, 22%, 13%, 9% and 6% respectively [6]. Currently, surgical resection is still the main method to prolong the survival time of lung cancer, but the invasion and metastasis of lung cancer is the biggest obstacle to improve the efficacy of the prognosis of lung cancer. For in-depth study of lung cancer malignant behavior and focus on comprehensive treatment of metastatic lung cancer, it is necessary to establish appropriate animal model to study lung cancer recurrence and metastasis and its comprehensive therapy.

Natural killer (NK) cell, also known as large granular lymphocytes, is an independent and non-specific immune cell. It has no MHC restriction to target cells recognition and destruction, and it can directly kill tumor cells and virus-infected target cells without antigen pre-sensitized [7,8]. It also can produce a large number of immune-active cytokines to enhance or expand its anti-tumor effect, which can be regarded as the first line of the host defense system [9]. With the development of tumor formation, malignant tumor cells and infiltrating immune cells interact and composed the tumor microenvironment. Most of studies published showed that a large number of immune cells infiltrating into tumor tissue played an important role in improving tumor prognosis [10,11]. The impact of NK cell receptor expression and function may be different caused by the interaction between NK cells and tumor in the tumor microenvironment. By exploring NK cells of the body and lung cancer microenvironment, discuss its distribution, receptor expression, functional status with lung cancer invasion, metastasis and prognosis, clarify the mechanism of NK cells involved in lung cancer microenvironment from the cellular and molecular levels.

Methods: NK cell infiltration number in different pathological types of human NSCLC was detected through the detection of NK cell surface receptors (CD56 and CD16) expression by immunofluorescence, and its correlation with the prognosis of patients with lung cancer was analyzed. The differences of NK cell receptors CD56 and CD16 mRNA expression between different pathological types of lung cancer was detected and verified by Real-time PCR. The SCID mouse transplanted tumor-lung metastasis model was produced by inoculated SCID mouse with human high-
metastatic large cell lung cancer cell lines L9981 and low-metastatic large cell lung cancer cell line NL9980. The lymphocytes of lung and spleen were extracted from the implanted tumor group and blank control group, the NK1.1⁺ CD3⁻ NK cells were separated and purified by flow cytometry from the mice lung and spleen lymphocytes in each group. The expression levels and difference of each group of mice lung and spleen NK cell surface receptor NKG2D, NKG2A and Ly49I from each group of mice lung and spleen were detected and analyzed by flow cytometry. NK1.1⁺ CD3⁻ NK cells in the spleen of the control group were co-cultured with high/low-metastatic lung cancer large cell lines L9981 and NL9980, then for the killing ability of NK1.1⁺ CD3⁻ NK cells to the different metastatic capacity of lung cancer cell was tested by flow cytometry. The expression levels of mRNA of NK cell surface receptor NK1.1, NKG2D, NKG2A and Ly49I were detected in the transplanted tumors in mice with different metastatic capacity lung cancer cell lines by real-time PCR.

**Results:** The infiltrated NK cells in the lung cancer tissue were mainly concentrated in the tumor stroma, constituting the tumor microenvironment. In NSCLC tissues, CD56⁺ NK cells with 1, 2 and 3 degree of infiltration were 44.0%, 22.6%, 33.3%, CD16⁺ NK cells were 45.2%, 22.6%, 32.1%, and CD56⁺ CD16⁺ NK cells were 45.2%, 23.8%, 31.0%, respectively. No significant difference was observed (p > 0.05) among NK cells with CD56 single positive, CD16 single positive and CD56CD16 double positive in the lung cancer tissue. The 1, 2 and 3 degree with CD5⁺ CD16⁺ NK cell infiltration were 24.3%, 32.4%, 43.2% in squamous cell carcinomas, and 62.2%, 16.2%, 21.6% in adenocarcinoma and 50.0%, 10.0%, 40.0% in large cell lung cancer, respectively. There was significant difference of CD5⁺ CD16⁺ NK cell infiltration degree between different pathological types of lung cancer (p=0.019). The expression of NK cells in squamous cell carcinoma was significantly higher than in adenocarcinoma and large cell carcinoma. The 1, 2 and 3 degree with NK cell infiltration were 48.1%, 3.7%, 48.1% in lung cancer patients with no history of smoking, were 42.1%, 31.7%, 26.3% in patients with smoking history. Smoking history of lung cancer patient is related to NK cell infiltration degree (p=0.011). The 1, 2 and 3 degree with NK cell infiltration were 60.6%, 18.4%, 21.1% in the T1-T2 cancer, and 42.1%, 31.7%, 43.5% in the T3-T4 cancer, respectively. A significant difference of NK cell infiltration degree was found between different size of the tumor (p=0.019). The survival time of lung cancer patient was positively related to NK cell infiltration degree in lung cancer. The more infiltration of NK cells were existed, the longer the survival time of patients did (p=0.030). Transplanted tumor-lung metastasis models were successful established in in SCID mic with high(L9981)/low(NL9980) metastatic human large cell lung cancer cell lines. The distant metastasis of the xenograft tumor was detected by fluorescence imaging in vitro. Compared with low-metastatic group (6.84×10⁶±3.26×10⁶), the lung metastases fluorescence value of mice in the high-metastatic group (30.97×10⁶±14.3×10⁶) was remarkably higher than that in low-metastatic group (p=0.035). NK1.1⁺ CD3⁻ NK cells in lungs of high-metastatic group (3.40±0.90) were significantly higher than that in spleen (0.10±0.06)(p=0.003). NK1.1⁺ CD3⁻ NK cells in spleen of high-metastasis group (0.10 ± 0.06) were significantly lower than that in low-metastasis group (1.66±0.82) and control group (3.80±2.05) (p=0.017, p=0.025). NKG2D expression level of NK cells from spleen in high-metastasis group (4.17±0.85) were remarkably lower than that in control group (7.80±2.67)(p=0.034) and low-metastasis group (6.00±0.96)(p=0.040). NKG2A expression level of NK cells from lung (5.13±2.36) was significantly higher than that from spleen (1.47±0.68) in high-metastatic group (p=0.007) . KG2A expression level of NK cells from lung in high-metastatic group
(5.13±2.36) were significantly higher than that in low-metastatic group (4.70±1.96) and control group (0.73±0.26) (p =0.000). Ly49I expression level of NK cells from lung in high-metastatic group (4.82±1.78) was remarkably upregulated than that in spleen (1.50±0.10) (p=0.003). The Ly49I expression level of NK cells from the lung (2.79±0.40) was significantly higher than that from the spleen (1.13±0.40) in control group (p=0.033). Ly49I expression level of the NK cells from the lung in high-metastatic group (4.82±1.78) and low-metastatic group (6.11±2.23) was significantly higher than that in control group (2.79±0.40) (p=0.000). Ly49I expression level of NK cells from the spleen in low-metastatic group (3.40±1.00) was remarkably high than that in high-metastatic group (1.50±0.10) (p=0.010) and control group (1.13±0.40) (p=0.004). NK1.1^CD3^-NK cells activated by IL-2 in vitro have significant cytotoxic activity to high/low metastatic lung cancer large cells. A significant difference was observed between different effector-target ratio (p=0.005, p=0.017). NK cells had higher cytotoxic activity to NL9980 than that to L9981 in the same effector-target ratio (p=0.035). In the transplanted tumors of tumor-bearing group, NKG2D expression level of NK cells in high-metastasis group was significantly higher than that in low-metastasis group (p=0.018), and the Ly49I expression level of NK cells in high-metastasis group was also remarkably higher than low-metastasis group (p=0.001). However, no significant difference of the NK1.1 and NKG2A expression level of NK cells was existed between high and low metastasis group (p>0.05).

**Conclusions:** (1) The number of NK cell infiltrating in lung cancer tissue is closely related to the pathological types, size of the primary cancer, smoking history and prognosis of the patients with lung cancer. (2) The activated receptor of NK cells is downregulated and inhibitory receptor of NK cells is upregulated in the transplanted tumor and the distant metastatic lesions of human large cell lung cancer cell lines, which might be the main reason leading to NK cell killing ability decreased. (3) A higher resistant to cytotoxic activity of NK cell exist in the human high-metastatic large cell lung cancer cell line L9981, which is much higher than that in the low-metastatic large cell lung cancer cell line NL9980. The variation of NK cell receptors might be useful in the prediction of tumor cell invasion and metastasis capacity, and further studies are needed to figure out the exact mechanism.

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**B89** Differential expression of OPN in lung cancer cell lines with different metastatic Potential and its role in migration and invasion

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**Background and Objective:** Lung cancer is the most common cause of cancer-related death in the world. Approximately 80% of all lung cancer patients are categorized as non–small-cell lung cancer (NSCLC). Most patients present with locally advanced or metastatic disease at the time of diagnosis. Unfortunately, so far, the mechanism of lung cancer metastasis is still not very clear.

Previous studies have shown that OPN modulates cell migration and invasion in several types of cancer cells. Elevated expression of OPN mRNA or protein were correlated with shortened survival in the patients with early-stage NSCLC. Moreover, Plasma OPN levels of early-stage NSCLC patients are gradually decreased after resection of the tumor and increased again when cancer recurrence was developed, OPN concentration is elevated in the plasma of advanced stage NSCLC patients, overexpression of OPN is associated with more aggressive phenotypes in human non-small cell lung cancer, which suggest OPN may modulate cell migration and invasion of NSCLC. However, the molecular mechanisms which determine expression of OPN in NSCLC are largely unknown.

Invadopodia are found in several types of invasive tumor cells and are associated with breaching tissue boundaries and they enable cancer invasion and tumor metastasis. It is unclear, however, whether the invadopodia involved in lung cancer cell migratory and/or invasive processor.

To further explore the role of OPN in regulation mechanism of lung cancer cells migration and invasion. We transfected NL9980 cells with OPN-PcDNA3.1 (+) vector, and treat A549 cells with ectogenic soluble OPN, then examined the expression of OPN both at mRNA and at protein level in those cells. Immunofluorescence was performed to investigate the effect of OPN on the regulation of invadopodia formation and FITC-gelatin degradation. Because the L9981 with the absent nm23-H1 expression, we hypothesize that nm23-H1 may suppress lung cancer cells migration and invasion by modulating OPN. We compare the OPN expression of human large cell lung cancer cell line NL9980 by nm23-H1 gene stable silencing with that of negative control cells.

To find out the presence of invadopodia in lung cancer cells and the regulation mechanisms of invadopodia, we carried out immunofluorescence and FITC-gelatin degradation assay to demonstrate the presence of invadopodia in L9981, NL9980 and A549 lung cancer cells. In addition, we examined the expression of osteopontin, cortactin, Arp2, and MMP-9 in NL9980 and NL9980-OPN. The result of our data indeed demonstrated that invadopodia are present in lung cancer cells A549 and L9981. Invadopodia enable polarized invasion of A549 and L9981 cells into the gelatin matrix in a time-dePendent manner. Moreover, A549 and NL9980 cells forced
overexpressing osteopontin (OPN) displayed an increase in the number of invadopodia and gelatinolytic activity accompany with elevated expression of cortactin, Arp2. In addition, OPN treatment might up-regulate the expression of cortactin, Arp2 and MMP-9 of A549 cells in a time-dependent manner. Furthermore, we found that forced overexpressing of OPN remarkably activate the Src, ERK1/2 phosphorylation. Our data presented here offer clear evidence that invadopodia participates in the process of migration and invasion of lung cancer cells, and OPN may improve the invadopodia formation by activate the Src, ERK1/2 pathway.

Methods: Western Blot and real-time quantitative PCR were used to determine the protein and mRNA expression levels of nm23-H1 and OPN gene in NL9980 and L9981 cells. For OPN forced overexpression, pcDNA3.1 (+)-OPN was transiently transfected into NL9980 via cationic liposome Lipofectamine 2000TM, Western Blot and real-time quantitative PCR were used to detect the efficiency of OPN protein and mRNA expression levels; the effect of forced OPN overexpression and soluble OPN treatment on the protein and mRNA expression of cortactin, Arp2 and MMP-9 gene were tested by Western Blot and real-time quantitative PCR. Src and Erk1/2 signaling pathway proteins were detected by western blot; Invasion assay and wound assay were used to observe the effects of OPN on migration and invasive ability in lung cancer cells in vitro. Invadopodia and the degradation of FITC-gelatin were observed by Fluorescence microscope. Western Blot and real-time quantitative PCR were used to detect the effect of stable silencing nm23-H1 gene on OPN Protein and mRNA expression levels in NL9980 cells.

Results: (1) OPN mRNA expression in high metastatic potential large cell lung cancer cell line L9981 was significantly higher than that in the low metastatic large cell lung cancer cell line NL9980 (17.2 ± 2.725 vs 1, P <0.01); (2) OPN protein expression in high metastatic potential large cell lung cancer cell line L9981 was significantly higher than that in low metastatic large cell lung cancer cell line NL9980 (0.593 ± 0.112 vs 0.018 ± 0.001, P <0.01); (3) The OPN-pcDNA3.1 (+) expression plasmid was successfully constructed and the NL9980-OPN and A549-OPN lung cancer cell lines were established by transient transfection NL9980 and A549 cell lines with the OPN-pcDNA3.1 (+) expression plasmid. (4) the mRNA expression of OPN gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (368.88 ± 21.34 vs 0, P = 0.00); the protein expression of OPN gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (0.911 ± 0.016 vs 0.214 ± 0.015, P <0.01); (5) the mRNA expression of cortactin gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (P <0.01); the mRNA expression of Arp2 gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (P <0.01); (6) the Protein expression of cortactin gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (0.792 ± 0.205 vs 0.344 ± 0.042), (P <0.01) and the protein expression of Arp2 gene in NL9980-OPN cell lines was significantly higher than that in the NL9980-control cell line (0.454 ± 0.064vs 0.18 ± 0.049, P <0.01); (7) exogenous soluble OPN significantly increased MMP-9 mRNA expression in A549 cell line time-dependently. (8) Exogenous soluble OPN can promote the protein expression of cortactin and Arp2 gene in A549 cells time-dePendently. (9) the p-Akt protein expression in NL9980-OPN lung cancer cell line were significantly higher than that in NL9980-control lung cancer cell lines (0.647 ± 0.067 vs 0.457 ± 0.061), (P
the p-Src protein expression in NL9980-OPN lung cancer cell line was significantly higher than that in NL9980-control lung cancer cell lines (1.253 ± 0.194 vs 0.92 ± 0.066), ($P < 0.05$); the p-p38 protein expression in NL9980-OPN lung cancer cell line were significantly higher than that in NL9980-control lung cancer cell lines (0.433 ± 0.078 vs 0.280 ± 0.056), ($P < 0.05$); the p-p42 / p44 protein expression in NL9980-OPN lung cancer cell line was significantly higher than that in NL9980-control lung cancer cell lines (0.960 ± 0.125 vs 0.687 ± 0.055), ($P < 0.05$); the in vitro migration ability of NL9980-OPN lung cancer cell lines was significantly higher than that of NL9980-control lung cancer cell lines ($P < 0.01$); the in vitro migration ability of A549-OPN lung cancer cell lines was significantly higher than that of A549-control lung cancer cell lines ($P < 0.01$); the in vitro invasive ability of NL9980-OPN lung cancer cell lines was significantly higher than that of NL9980-control lung cancer cell lines ($P < 0.01$); the in vitro invasive ability of A549-OPN lung cancer cell lines was significantly higher than that of A549-control lung cancer cell lines ($P < 0.01$); No FITC-gelatin degradation was found in NL9980 lung cancer cell line after culture 12h, while L9981 and A549 cells displayed FITC-gelatin degradation. Arp2-enriched and cortactin-enriched dot-like invadopodia were observed at the periphery in the clear zone area of L9981 and A549 cells. Forced OPN over-expression can promote the degradation of FITC-gelatin in NL9980 time-dependently and Arp2-enriched dot-like invadopodia were observed at the periphery in the clear zone area. Compared with the control group, exogenous soluble OPN can significantly promote the degradation of FITC-gelatin of A549 cells ($P < 0.05$); The NL9980-nm23-H1-KD cell lines was successfully established, which with stable nm23-H1 gene silence; the OPN mRNA expression in NL9980-nm23-H1-KD cell lines was significantly higher than that in NL9980-control cell lines (4.95 ± 0.02 vs 1 ± 0.02), ($P = 0.000$); the OPN mRNA expression in L9981-nm23-H1-EGFP cell lines was significantly lower than that in L9981-EGFP cell lines (0.840 ± 0.032 vs 5.70 ± 0.21), ($P < 0.01$); the OPN protein expression in NL9980-nm23-H1-KD cell lines was significantly higher than that in NL9980-control cell lines (0.630 ± 0.088 vs 0.193 ± 0.032), ($P < 0.01$); the OPN protein expression in L9981-nm23-H1-EGFP cell lines was significantly lower than that in L9981-EGFP cell lines (0.173 ± 0.044 vs 0.463 ± 0.155), ($P < 0.01$); the in vitro invasive ability of NL9980-nm23-H1-KD cell lines was significantly higher than that of NL9980-control cell lines ($P < 0.01$).

**Conclusion:** (1) Overexpression of OPN mRNA and protein were existed in high metastatic potential lung cancer cell line L9981, and forced OPN overexpression and exogenous soluble OPN can significantly increase the expression of cortactin, Arp2, and MMP-9 in lung cancer cell lines. (2) Forced OPN overexpression can activate the Src, ERK1 / 2 signaling pathway in low-metastatic large cell lung cancer cell line NL9980 and significantly increase the in vitro migration and invasion ability of NL9980 and A549 cell lines; (3) Both forced OPN overexpression and exogenous soluble OPN can significantly induce the formation of invadopodia in NL9980 and A549 cell lines; (4) nm23-H1 gene can significantly decrease the OPN gene mRNA and protein expression in the high metastatic potential human large cell lung cancer cell line L9981, while nm23-H1 gene silencing can significantly increased the OPN mRNA and protein expression in the low metastatic potential human large cell lung cancer cell line NL9980 and give its high metastatic potential; (5) nm23-H1 gene can
inhibit and/or reverse the invasive and metastatic potential of human lung cancer by regulating OPN gene expression and it’s downstream genes and signal pathways.

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Separation of tetraspanin CD151 from its integrin partner α3β1 reflects an altered migratory state and predicts prostate cancer progression.

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The dysregulation of cell migration enables tumor cells to escape their tissue of origin and disseminate. Since cancer-related deaths are primarily caused by the dissemination of tumor cells, mechanisms of migration are both a target for therapy and an indicator of disease progression. The regulation of cell adhesion is widely recognized as a rate-limiting step in metastasis but how tumor cells achieve dynamic control over their adhesion receptors is poorly understood. During an analysis of prostate cancer progression we discovered that α3β1 expression is reduced and that its tetraspanin partner, CD151, is not “integrin-free”. We were able to detect integrin-free CD151 using antibodies specific to the integrin-binding domain of CD151. Dual staining of tumor tissue and normal tissue from prostate cancer patients for total CD151 and integrin-free CD151 revealed that the appearance of integrin-free CD151 corresponds with poor-patient outcome. In fact, the detection of integrin-free CD151 is an independent predictor of prostate cancer progression. Surprisingly, the clustering of integrin-free CD151 immobilizes tumor cells in vivo and prevents metastasis suggesting that the ability of CD151 to control migration does not depend on its α3β1 integrin partner. Indeed, integrin-free CD151 is now associated with non-integrin partners through which it can regulate tumor cell motility.

These observations demonstrate that the appearance of integrin-free CD151 reflects the disruption of the CD151/α3β1/laminin axis and thereby reveals an altered migratory ability in tumor cells. This has clinical as well molecular implications. Integrin-free CD151 can be used as a molecular indicator of disease progression and assist in the distinction between indolent (benign) and advanced disease. In addition, the identification of new CD151 partners can provide new therapeutic targets to inhibit the motility of tumor cells that have undergone this change in migratory status. A preliminary evaluation identified a similar appearance of integrin-free CD151 in cancers derived from other tissues, suggesting that this change in molecular status is broadly applicable to most solid tumors.
Notes
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