SPANISH NATIONAL CANCER RESEARCH CENTRE

CNIO FRONTIERS MEETINGS 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Madrid 28 — 30 September 2015

Organisers

David Lyden
Weill Cornell Medical College,
New York, US

Yibin Kang
Princeton University, New Jersey, US

Gemma Alderton
Nature Reviews Cancer, London, UK

Victoria Aranda
Nature Medicine, New York, US

Li-kuo Su
Cancer Cell, Cambridge, US

Héctor Peinado
CNIO, Madrid, Spain

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Instituto de Salud Carlos III

cnio Centro Nacional de Investigaciones Oncológicas

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Summary

SPANISH NATIONAL CANCER RESEARCH CENTRE
CNIO FRONTIERS MEETINGS 2015

Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Programme

CNIO Frontiers Meeting
Metastasis Initiation: Mechanistic Insights and Therapeutic Opportunities
September 28-30, 2015

cnio Centro Nacional de Investigaciones Oncológicas

CNIO Frontiers Meeting
Metastasis Initiation: Mechanistic Insights and Therapeutic Opportunities
September 28-30, 2015

Venue: Spanish National Cancer Research Centre (CNIO) Auditorium

Chairpersons:

David Lyden (Weill Cornell Medical College)
Yibin Kang (Princeton University)
Gemma Alderton (*Nature Reviews Cancer*)
Victoria Aranda (*Nature Medicine*)
Li-Kuo Su (*Cancer Cell*)
Héctor Peinado (CNIO**)**

Rationale:
The conference will focus on recent progress in understanding how metastasis is initiated and the molecular mechanisms underlying early events in metastatic progression. Some of the subjects discussed will be:

- Cell Fate Regulation, Stem Cells And Metastasis
- Circulating Factors and Their Role in Metastasis (i.e., exosomes, CTCs, DNA)
- Pre-metastatic Niche Formation
- Metastatic Organotropism
- Biomarkers and Treatment of Early Metastasis
- New Technologies Developed to Detect Metastatic Niches

Sunday September 27th

20:00-21:30 **Welcome cocktail for Speakers & Participants**
Venue: Hotel EXE Puerta Castilla
(Paseo de la Castellana 191 - 28046 Madrid España - +34 914531900)
http://www.hotelexepuertacastilla.com/

Monday September 28th

09:00-09:15 **Opening Remarks**
***David Lyden**, Weill Cornell Medical College, New York, US*
***Yibin Kang**, Princeton University, New Jersey, US*

09:15-10:15 **Topic I: Cell Fate Regulation, Stem Cells and Metastasis**
Chairperson: Yibin Kang, Princeton University

(09:15-09:45) Genes and Pathways That Link Mammary Gland Stem Cell Regulation, Tumor Initiation and Metastatic Properties
***Yibin Kang**, Princeton University, New Jersey, US*

(09:45-10:15) Breast Cancer Stem Cell State Transitions During Metastasis
***Max Wicha**, University of Michigan, US*

10:15-10:45 **Group picture and Coffee Break (Social Room)**

10:45-11:45 **Topic II: Epithelial-to-Mesenchymal Transition**
Chairperson: Yibin Kang, Princeton University

(10:45-11:15) Cellular Plasticity in Cancer: Driving Force and Therapeutic Target
***Thomas Brabletz**, University Erlangen-Nuernberg, Germany*

(11:15-11:45) Evolutionary pressure to maintain the epithelial phenotype: impact on metastasis initiation
***Angela Nieto**, Neuroscience Institute of Alicante, Spain*

11:45-12:45 **Topic III: Circulating Factors: Microvesicles and Exosomes**
Chairperson: Yibin Kang, Princeton University

(11:45-12:15) Role of Tumor-secreted Exosomes Educating the Tumor Microenvironment
***Héctor Peinado**, CNIO, Madrid, Spain*

(12:15-12:45) Extracellular miRNA and Metabolic Adaptation of Cancer-hosting Niche
***Emily Wang**, City of Hope Beckman Research Institute, Duarte, US*

12:45-14:00 **Lunch Break (Cafeteria)**

14:00-15:00 **Topic III: Circulating Factors: Circulating Tumor Cells/Platelets/Circulating DNA and RNA**
Chairperson: Héctor Peinado, CNIO

(14:00-14:30) Mechanisms and Inhibition of Breast Cancer Brain Colonization
***Brunie Felding-Habermann**, The Scripps Research Institute, La Jolla, US*

(14:30-15:00) Circulating Tumor Cells: Biology and Clinical Relevance
***Klaus Pantel**, University Medical Center Hamburg-Eppendorf, Germany*

15:00-15:30 **Coffee Break (Social Room)**

Monday September 28th

15:30-16:30 **Short Talks**

- Mesenchymal Status Promotes Metastatic Colonization via a Cancer Cell-stroma Crosstalk which Uncouples EMT and Stemness
  ***Ilaria Malanchi**, Cancer Research UK, London Research Institute, UK*
- In vivo Imaging Reveals Extracellular Vesicle-mediated Phenocopying of Metastatic Behavior
  ***Anoek Zomer**, Hubrecht Institute, Utrecht, Netherlands*
- The E3-ligase E6AP Represses Breast Cancer Metastasis through Regulation of ECT2-Rho-GTPases Signalling
  ***Mariam Mansour**, PeterMac Callum Cancer Centre, Melbourne, Australia*
- N-Myc and STAT interactor: A regulator of tumor progression and EMT
  ***Rajeev Samant**, University of Alabama at Birmingham, US*

20:30 Speakers dinner:
Venue: Goizeko-Kabi Restaurant.
Comandante Zorita street 37, Madrid

Tuesday September 29th

09:00-10:00 **Topic IV: Pre-Metastatic Niche**
Chairperson: David Lyden, Weill Cornell Medical College

(09:00-09:30) ECM Remodeling During Cancer Progression
***Janine Erler**, Biotech Research & Innovation Centre, University of Copenhagen, Denmark*

(09:30-10:00) How Lymphatic Endothelial Cells Help Transform the Draining Lymph Node into a Pre-metastatic Niche
***Melody Swartz**, University of Chicago, US*

10:00-10:45 **Coffee Break (Social Room)**

10:45-12:15 **Topic V: Disseminated, Dormant and Metastasis-Initiating Tumor Cells**
Chairperson: David Lyden, Weill Cornell Medical College

(10:45-11:15) The Origins of Disseminated Cancer Cells and Their Role in Dormancy and Metastatic Reactivation
***Julio Aguirre-Ghiso**, Mount Sinai Medical Center, New York, US*

(11:15-11:45) Where the Wild Things Are: The Perivascular Niche Regulates Disseminated Tumor Cell Dormancy and Drug Resistance
***Cyrus Ghajar**, Fred Hutchinson Cancer Research Center, Seattle, US*

(11:45-12:15) The Natural History of Metastasis: Why Does Adjuvant Chemotherapy Work?
***Ben Stanger**, University of Pennsylvania Perelman School of Medicine*

Tuesday September 29th

12:15-13:15 **Topic VI: Organ-Specific Metastasis and Micrometastatic Disease**
Chairperson: David Lyden, Weill Cornell Medical College

(12:15-12:45) Metastasis Initiating Cells, Niches and Vital Pathways
***Joan Massagué**, Memorial Sloan Kettering Cancer Center, New York, US*

(12:45-13:15) Tumor-derived Vesicles Initiate Organtropic Niches
***David Lyden**, Weill Cornell Medical College, New York, US*

13:15-14:15 **Lunch Break (Cafeteria)**

14:15-15:15 **Topic VII: Imaging Early Metastatic Events**
Chairperson: Erik Sahai, London Research Institute, UK

(14:15-15:15) Modelling Cancer Cell Invasion in Complex Environments
***Erik Sahai**, London Research Institute, UK*

(14:45-15:15) Regulation of Tumor Immunity by Extracellular Vesicles: Analysis in Context
***Mikael Pittet**, Harvard University, Cambridge, US*

15:15-16:15 **Topic VIII: Targeting Metastasis**
Chairperson: Erik Sahai, London Research Institute, UK

(15:15-15:45) Targeting the HIF Pathway Therapeutically to Treat Metastasis
***Amato Giaccia**, Stanford School of Medicine, US*

(15:45-16:15) Identification and Therapeutical Targeting of Metastasis-Initiating-Cells in Human Oral Squamous Cell Carcinoma
***Salvador Aznar Benitah**, Institute for Research in Biomedicine, Barcelona, Spain*

16:15-16:45 **Coffee Break (Social Room)**

16:45-17:45 **Short Talks**

- Cancer: Opening LOX to metastasiss
  ***Thomas Cox**, Biotech Research & Innovation Centre (BRIC), Copenhagen, Denmark*
- LIPG Endothelial Lipase is Essential for Breast Cancer Growth and Metastasis Through Control of Lipid Supplies
  ***Roger Gomis**, Institute for Research in Biomedicine, (IRB) Barcelona, Spain*
- Endothelial Selectin-Mediated Recruitment of Myeloid Cells is Required for Tumor Cell Extravasation and Metastasis
  ***Lubor Borsig**, University of Zurich, Switzerland*
- Molecular Regulation of the Critical Steps to Initiate Brain Metastasis
  ***Manuel Valiente**, Spanish National Cancer Research Centre, Madrid, Spain*

17:45 **Wine and Cheese – Poster Discussion (Social Room)**

Wednesday September 30th

09:00-10:30 **Topic IX. Modeling metastasis**
Chairperson: Cyrus Ghajar, Fred Hutchinson Cancer Research Center, Seattle, US

(09:00-09:30) Cooperative events in metastasis identified through zebrafish models
***Richard Mark White**, Memorial Sloan Kettering Cancer Center, New York, US*

(09:30-10:00) Understanding metastatic predisposition through genome-wide screens for metastasis susceptibility genes
***Kent W. Hunter**, Center for Cancer Research, National Cancer Institute, Bethesda, US*

(10:00-10:30) Non-invasive imaging of neo-lymphangiogenesis for the identification of metastatic niches and anticancer agents in melanoma
***María S. Soengas**, Spanish National Cancer Research Centre, Madrid, Spain*

10:30-11:30 **Short Talks**

- Astrogliosis and neuroinflammation are instigated in a mouse model of spontaneous melanoma brain metastasis
  ***Neta Erez**, Tel Aviv University, Israel*
- Tracking rare and dynamic events in vivo at high resolution using multimodal correlative microscopy
  ***Jacky Goetz**, INSERM, Strasbourg, France*
- Chemotherapy alters the natural history of metastatic progression
  ***Nicole Aiello**, University of Pennsylvania, Philadelphia, US*
- Genome-wide in vivo screening identifies novel microenvironmental regulators of metastatic colonisation
  ***Louise Van Der Weyden**, Wellcome Trust Sanger Institute, Cambridge, UK*

11:30-12:00 **Coffee Break (Social Room)**

12:00-13:00 **Meet the Journal Editors**
***Gemma Alderton**, Nature Reviews Cancer, London, UK*
***Victoria Aranda**, Nature Medicine, New York, US*
***Li-Kuo Su**, Cancer Cell, Cambridge, US*
***Alexia-Ileana Zaromytidou**, Nature Cell Biology, London, UK*

13:00-13:15 **Closing Remarks**
***Yibin Kang**, Princeton University, US*
***David Lyden**, Weill Cornell Medical College, New York, US*

Talks: 25'+5' discussion
Short Talks: 10'+5' discussion

Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic I

Cell Fate Regulation, Stem Cells and Metastasis

Chairperson: Yibin Kang, Princeton University

Monday September 28th

Genes and Pathways that Link Mammary Gland Stem Cell Regulation, Tumor Initiation and Metastatic Properties

Yibin Kang,
Princeton University, Molecular Biology, Princeton, NJ, US

Metastasis represents the most devastating stage of cancer progression and is responsible for most of cancer-related death. 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, early oncogenic events, as well as the stresses tumor cells endure when they encounter different microenvironments and therapeutic treatments. In mammary gland development, transcription factor ΔNp63 and Snail maintain mammary gland stem cell activity while Elf5 and Gata3 drive luminal differentiation. Altered activities of these cell fate regulators influence the development of breast cancer subtypes and their metastatic capabilities. In addition to these cell-intrinsic regulators, epithelial-stromal interactions mediated by the Notch pathway ligands and receptors also play key roles in regulating mammary gland development and differentiation, and shape the acquisition of metastatic capabilities both in the primary tumor and in distant organs. Tumor-specific stem cell survival factors such as MTDH additionally confer growth advantage under oncogenic stress conditions in primary tumors and during metastasis, and represents ideal therapeutic targets to thwart metastatic progression while sparing normal tissues.

Breast Cancer Stem Cell State Transitions During Metastasis

Max Wicha,
University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan, US

Tumor cellular heterogeneity represents one of the greatest challenges to the development of molecularly targeted therapeutics. In addition to genetic heterogeneity resulting from mutation and clonal selection, there is increasing evidence for an important role of epigenetic regulation in generating cellular heterogeneity and therapeutic resistance. This epigenetic heterogeneity recapitulates gene regulation during cellular differentiation. At the apex of this hierarchy are "cancer stem cells" (CSCs) that are characterized by their ability to self-renew, as well as to generate the populations constituting the tumor bulk. CSCs mediate tumor metastasis as well as resistance to cytotoxic chemotherapy and radiation areas. CSCs themselves may exhibit intratumor molecular heterogeneity generated through both genetic and epigenetic mechanisms. We have demonstrated that human breast cancer CSCs exist in alternative states characterized by expression by different molecular markers. Mesenchymal EMT-like CSCs characterize as CD44+/CD24- are largely quiescent but highly invasive. In contrast, epithelial (MET) CSCs are characterized by expression of aldehyde dehydrogenase (ALDH) are proliferative and capable of generating more differentiated cells constituting the tumor bulk. The plasticity of CSCs to transition between EMT and MET-like states is critical to the process of metastasis.

In order to examine the genetic and epigenetic changes in CSCs, we have developed microfluidic technologies to isolate circulating tumor cells (CTCs) from patients with metastatic breast cancer. While cells displaying CSC markers constitute 1%-5% of primary tumors, approximately 50% of CTCs express CSC markers. Furthermore, a substantial proportion of these cells display markers of EMT. In addition to circulating single cells, there is increasing evidence that tumor metastasis may be mediated by clusters of CTCs. Cell-to-cell interactions within these clusters may facilitate survival of CSCs as well as their seeding at metastatic sites. We have developed microfiltration techniques that allows for separation of single and clusters of CTCs and demonstrated cellular heterogeneity within these clusters. Furthermore, we have utilized multiplex PCR (Fluidigm) to molecular interrogate CTCs at single cell resolution. A number of agents designed to target CSCs have now entered clinical trials. The development of technologies to isolate and molecularly interrogate CTCs from patients on these trials should facilitate clinical development of these CSC targeting agents.

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Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic II

Epithelial-to-Mesenchymal Transition

Chairperson: Yibin Kang, Princeton University

Monday September 28th

Cellular Plasticity in Cancer: Driving Force and Therapeutic Target

Thomas Brabletz,
Nikolaus-Fiebiger Center for Molecular Medicine, University Erlangen-Nuremberg, Germany

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

We described that the EMT-activator and transcriptional repressor ZEB1 is a crucial determinant of cellular plasticity. At molecular level, ZEB1 is linked in a double negative feedback loop with the miR-200 family and miR-203, which are strong inducers of epithelial differentiation. Thus aberrant ZEB1 expression stabilizes EMT and stemness, thereby promoting dissemination, metastasis and drug resistance of cancer cells. We have validated the findings, by showing that a depletion of ZEB1 in the KPC-mouse model of pancreatic cancer counteracts tumor cell plasticity and metastasis. Moreover we detected that ZEB1 controls the Notch pathway and directly cooperates with the Hippo-pathway effector YAP in driving aggressive cancer types.

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 indicate that breaking the ZEB1 - miR-200 feedback loop is a treatment option for fatal tumors such as pancreatic cancer.

Evolutionary Pressure to Maintain the Epithelial Phenotype: Impact on Metastasis Initiation

Angela Nieto,
Neuroscience Institute of Alicante, Spain

Epithelial homeostasis is crucial to maintain tissue architecture, and therefore, it needs to be tightly regulated in the adult. By contrast, embryonic and cancer cells show a high degree of epithelial plasticity required for proper morphogenesis and for the completion of the metastatic cascade, respectively. Epithelial plasticity is governed by the so called epithelial to mesenchymal transition (EMT), which is fundamental for cell migration in embryos and for cell delamination from primary tumors. Upon reaching their final destinations, embryonic and cancer cells revert to the epithelial phenotype through the inverse process (MET) to form organs or metastases. I will present new data to indicate that evolution has favored the maintenance of the epithelial phenotype by attenuating the function of the novel EMT inducer Prrx1. I will discuss the implications that this mechanism can have in cell behavior and, in particular, in metastatic colonization.

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Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic III

Circulating Factors: Microvesicles and Exosomes

Chairperson: Yibin Kang, Princeton University

Monday September 28th

Role of Tumor-Secreted Exosomes Educating the Tumor Microenvironment

Héctor Peinado,
Spanish National Cancer Research Centre, Madrid, Spain

Cancer is a systemic disease, while most of the research effort has been focused on analyzing the primary tumor, there is a lack of information on how the tumor microenvironment influences metastasis. The importance of the microenvironment in metastasis is now fully acknowledged. Prominent roles for stromal cells, such as fibroblasts, endothelial cells, lymphatic endothelial cells, bone marrow-derived cells, soluble factors and secreted vesicles have been established. Exosomes are secreted vesicles carrying lipids, proteins, RNA and DNA molecules. By carrying these molecules, and facilitating their cell-to-cell transfer, exosomes can modulate the behavior of resident cells that can impact disease progression. Exosomes can serve as vehicles for horizontal transfer of proteins, RNA and DNA to the surrounding cells, thus promoting additional modifications in the tumor and metastatic microenvironments. Our studies in metastatic melanoma demonstrated that tumor exosomes are a major tumor-derived factor that acts systemically to promote bone marrow-derived cells (BMDCs) recruitment to the tumor and metastatic microenvironments. We showed that exosome secretion by melanoma cells influences BMDC mobilization and recruitment to pre-metastatic and metastatic niches, thus promoting metastasis in a process that we have termed “education”. Our novel studies suggest that tumor exosomes can fuse specifically to stromal cells within the metastatic microenvironments. Specifically, we have analyzed the role of tumor-secreted factors in lymph node metastasis and local lymph node remodeling. We are currently analyzing circulating exosomes in the lymphatic fluid of cancer patients as a new approach to define new markers involved in tumor metastasis. Our data suggest that tumor-secreted exosomes act locally and systemically remodeling the lymphatic system favoring metastasis.

Extracellular miRNA and Metabolic Adaptation of Cancer-Hosting Niche

Shizhen Emily Wang,
City of Hope Beckman Research Institute, Duarte, US

Extracellular microRNAs (miRNAs) that can be detected in the circulation are novel mediators of intercellular communication and are considered emerging biomarkers for human diseases. Using cell-secreted microvesicles (e.g., exosomes) as vehicles, miRNAs secreted by cancer cells can travel to and enter various types of niche cells in primary and pre-metastatic tumor microenvironments. Upon entering niche cells, miRNAs regulate gene expression to prepare the niche for cancer progression. Our lab focuses on defining the roles of breast cancer (BC)-secreted miRNAs in adapting local and distal niche cells and tissues in cancer progression and metastasis. Through de novo sequencing of circulating small RNAs in the pre-treatment sera of stage II–III BC patients we identified circulating miR-122 as a biomarker for cancer progression to metastatic disease. Subsequent mechanistic studies revealed an important function of BC-secreted miR-122 in downregulating glucose metabolism in pre-metastatic niche cells in the brain and lungs. This leads to a nutrient reallocation between cancer and host cells, which increases nutrient availability to cancer cells and facilitates cancer progression. Our lab is currently exploring additional mechanisms through which BC-derived, extracellular miRNAs contribute to the metabolic reprogramming of non-cancerous cells in the tumor microenvironment as well as novel therapeutic strategies targeting cancer-derived extracellular miRNAs for their function in cancer–host communication.

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Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic III

Circulating Factors: Circulating Tumor Cells/Platelets/Circulating DNA and RNA

Chairperson: Héctor Peinado, CNIO

Monday September 28th

Mechanisms and Inhibition of Breast Cancer Brain Colonization

Brunie Felding-Habermann,
The Scripps Research Institute, La Jolla, US

Brain metastases are frequent in breast cancer patients and commonly fatal. Thus, strategies for prevention are urgently needed. However, molecular mechanisms of tumor cell brain colonization are poorly understood. We demonstrate that breast cancer cell expressed tissue factor promotes seeding of brain metastases from the blood stream by differentially contributing to intravascular tumor cell survival and crossing of the blood-brain barrier. Single dose treatments with antibodies inhibiting discrete human tissue factor functions identify two distinct mechanisms through which tissue factor supports brain colonization. First, blocking tissue factor-induced coagulation reduces tumor cell retention and survival within the cerebral microvasculature, and thereby prevents brain metastasis in mouse models. Second, we show that tumor cells reside within the cerebral microvasculature for up to five days before they cross the blood brain barrier, a step we identify as dependent on tissue factor signaling. This activity promotes interaction between tissue factor and tumor cell integrins, adhesion receptors that are functionally modified in this process to support invasion of the blood brain barrier and brain tissue. *In situ* analysis of clinical specimens reveals tissue factor expression in human primary breast carcinomas and their matched brain metastatic lesions indicating a clinical relevance of tissue factor, even at advanced stages of progression to brain metastasis. Our results identify tissue factor as a multifactorial mediator of tumor cell brain colonization and as a target for prevention of breast cancer brain metastasis.

Circulating Tumor Cells: Biology and Clinical Relevance

Klaus Pantel,
University Medical Center Hamburg-Eppendorf, Germany

Sensitive methods have been developed to capture circulating tumor cells (CTCs) in the peripheral blood and disseminated tumor cells (DTCs) in the bone marrow at the single cell level in cancer patients (Alix-Panabieres & Pantel, Nature Rev Cancer 2014). The analysis of CTCs may provide clinically relevant information as "liquid biopsy" (Pantel & Alix-Panabieres, Cancer Res., 2013; Pantel et al., Nature Med. 2013; Heitzer et al., Genome Med. 2013; Schwarzenbach et al., Nature Rev. Clin. Oncol., 2014). Moreover, analysis of DTCs provide new insights into the process of "cancer dormancy" (Uhr & Pantel, PNAS, 2011, Lu et al., Cancer Cell, 2011; LeBleu et al., Nat. Cell Biol., 2014) and also lead to the discovery of new molecules relevant to the biology of metastasis such as the putative metastasis-suppressor RAI2 (Werner et al., Cancer Discovery, 2015).

For clinical use, blood analyses are much more acceptable than invasive bone marrow aspirations. 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 might therefore overcome this important limitation. Although the majority of CTC studies have been performed on patients with carcinomas, CTCs have been also recently detected in the peripheral blood of patients with primary brain tumors (Mueller et al., Science Transl. Med., 2014). CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy to identify therapeutic targets or mechanisms of resistance on metastatic cells such as mutations in KRAS or expression of the androgen receptor variant 7 (Alix-Panabieres & Pantel, Nature Rev Cancer 2014). Metastatic cells might have unique characteristics that can differ from the bulk of cancer cells in the primary tumor currently used for stratification of patients to systemic therapy. 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 and might serve as surrogate marker for response to therapy (Bidard et al., Lancet Oncol. 2014; Scher et al., J. Clin Oncol. 2015). In the context of recent success in antibody-mediated blockade of immune checkpoint control molecules, expression of the PD-L1 on CTCs (Mazel et al, Mol. Oncol. 2015) might be of interest as potential predictive marker. Functional characterization using specialized in vitro and in vivo test systems has started (Bacelli et al, Nat. Biotech. 2013; Hodgkinson et al, Nat. Med. 2014; Cayrefourcq et al., Cancer Res. 2015), which might provide novel insights into the biology of CTCs and serve as models for drug testing.

In conclusion, the molecular and functional analysis of CTCs 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).

SHORT TALK

Mesenchymal Status Promotes Metastatic Colonization Via a Cancer Cell-Stroma Crosstalk Which Uncouples EMT and Stemness

Yaiza del Pozo Martin[1], Danielle Park[2], Fernando Calvo[4], Probir Chakravarty[3], Stefanie Derzsi[2], Erik Sahai[2] and **Ilaria Malanchi[1*]**

[1] Tumour Host Interaction Laboratory
[2] Tumour Cell Biology Laboratory
[3] Bioinformatics and BioStatistics Team London Research Institute, Cancer Research UK, London
[4] Tumour Microenvironment Team, Division of Cancer Biology, The Institute of Cancer Research, London

During metastatic colonisation tumour cells must establish a favourable microenvironment or niche that will sustain their growth. However, both the temporal and molecular details of this process are poorly understood. Here we find that metastatic initiating cells (MICs) exhibit high niche activation ability as a result of Thrombospondin 2 (THBS2) expression, which prompt lung fibroblasts activation. MICs are also characterised by a mesenchymal phenotype. Importantly, inhibiting the mesenchymal phenotype of MICs, by blocking the epithelial to mesenchymal transition (EMT)-associated kinase AXL, during the early stages of cancer cell infiltration, reduces THBS2 secretion, niche activation ability, and consequently metastatic establishment.

Subsequently, cancer cells start expanding at the target site and revert to a more epithelial phenotype. Interestingly, the activated fibroblasts of the newly formed metastatic niche trigger the reversion of cancer cell mesenchymal phenotype without compromising self-renewal. Preventing this phenotypic reversion by enhancing AXL expression during colonization block metastasis. In summary, we demonstrate that during metastatic colonization the two events of niche activation and EMT are functionally linked. However, in this context, EMT becomes uncoupled from the 'stemness' required for metastatic growth. Further, once fibroblasts within the niche are activated they promote the mesenchymal to epithelial transition (MET) of tumour cells, leading to the outgrowth of metastases with similar epithelial characteristics and stromal cell networks to their original primary tumours.

SHORT TALK

In vivo Imaging Reveals Extracellular Vesicle-Mediated Phenocopying of Metastatic Behavior

Anoek Zomer[1], Carrie Maynard[1], Frederik Johannes Verweij[2], Alwin Kamermans[1], Ronny Schäfer[1], Evelyne Beerling[1], Raymond Michel Schiffelers[3], Elzo de Wit[1], Jordi Berenguer[4], Saskia Inge Johanna Ellenbroek[1], Thomas Wurdinger[4,5], Dirk Michiel Pegtel[2], Jacco van Rheenen[1]

[1]Hubrecht Institute-KNAW & University Medical Center Utrecht, Utrecht, the Netherlands
[2]Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands
[3]Laboratory Clinical Chemistry & Haematology, University Medical Center Utrecht, Utrecht, the Netherlands
[4]Department of Neurosurgery, VU University Medical Center, Amsterdam, the Netherlands
[5]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, US

Most cancer cells release heterogeneous populations of extracellular vesicles (EVs) containing proteins, lipids and nucleic acids that reflect, at least in part, the cell of origin. Accumulating data obtained in vitro has demonstrated that the molecules packaged in EVs can be functionally transferred into a variety of recipient cells affecting their gene expression and behavior. However, similar in vivo experiments remain challenging, since cells that take up EVs cannot be discriminated from non-EV-receiving cells. To visualize the functional transfer of molecules packaged in EVs in real-time in vivo, we developed a Cre recombinase-based method, providing further opportunities to study in detail the distribution of EVs and biological relevance of EV transfer between various cells and tissues. We show that EVs released by malignant tumor cells are taken up by less malignant tumor cells located within the same and within distant tumors, and that these EVs carry mRNAs involved in migration and metastasis. By intravital imaging we show that the less malignant tumor cells that take up EVs displayed enhanced migratory behavior and metastatic capacity. The data presented here are consistent with the idea that tumor cells do not act autonomously, but can share proteins and nucleic acids with other tumor cells, locally and systemically. These results shed light on the mutual influence of cancer cells, and draw a new perspective on the complexity of intercellular communication in diseases such as cancer.

SHORT TALK

The E3-ligase E6AP Represses Breast Cancer Metastasis through Regulation of ECT2-Rho-GTPases Signalling

Mariam Mansour[1], Sue Haupt[1], Ai-Leen Chan[1], Nathan Godde[1], Alexandra Rizzitelli[1], Sherene Loi[1], Franco Caramia[1], Siddhartha Deb[2], Elena A. Takano[2], Mark Bishton[1], Cameron Johnstone[1], Yarra Levav-Cohen[1], Yong-Hui Jiang[3], Alpha S. Yap[4], Stephen Fox[2,5], Ora Bernard[6], Robin Anderson[1,5], and Ygal Haupt[1,5,7]

[1] Research Division, Peter MacCallum Cancer Centre, East Melbourne, Australia
[2] Pathology Department, Peter MacCallum Cancer Centre, East Melbourne, Australia
[3] Division of Medical Genetics, Department of Pediatrics and Neurobiology, Duke University School of Medicine, Durham, North Carolina, USA
[4] Division of Cell Biology and Molecular Medicine, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland, Australia
[5] Sir Peter MacCallum Department of Oncology, the University of Melbourne, Victoria, Australia
[6] St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia
[7] Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia

Metastatic disease is the major cause of breast cancer related death and despite many advances, current therapies are rarely curative. Tumor cell migration and invasion require actin cytoskeletal reorganisation, to endow cells with capacity to disseminate and initiate the formation of secondary tumors. However, it is still unclear how these migratory cells colonise distant tissues to form macrometastases. The E6-associated protein, E6AP, acts both as an E3 ubiquitin-protein ligase and as a coactivator of steroid hormone receptors. We report that E6AP suppresses breast cancer invasiveness, colonisation and metastasis in mice and in breast cancer patients, loss of E6AP associates with poor prognosis, particularly for with basal breast cancer. E6AP regulates actin cytoskeletal remodelling via regulation of Rho-GTPases, acting as a negative regulator of ECT2, a GEF required for activation of Rho-GTPases. E6AP promotes ubiquitination and proteasomal degradation of ECT2 for which high expression predicts poor prognosis in breast cancer patients. We conclude that E6AP suppresses breast cancer metastasis by regulating actin cytoskeleton remodelling through the control of ECT2 and Rho-GTPase activity. These findings establish E6AP as a novel suppressor of metastasis and provide a compelling rationale for inhibition of ECT2 as a therapeutic approach for patients with metastatic breast cancer.

SHORT TALK

N-Myc and STAT Interactor: A Regulator of Tumor Progression and EMT

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EMT is one of the key phenomena underlying metastatic colonization. Thus it is critical to define the triggers and regulators of EMT. NMI is an inducible protein that interacts with several key transcription factors that critically impact tumor progression. We discovered that expression of NMI is down-regulated in metastatic breast tumors. Silencing NMI expression from epithelial-like breast cancer cells enabled cells to assume a mesenchymal-like phenotype. Our investigations revealed that this mesenchymal transition was facilitated by decreased STAT5 signaling that caused concomitant reduction in SMAD7 expression and manifestation of a TGFβ-driven EMT program.

Autophagy is a determinant of cellular survival through dormancy as well as cytotoxic drug insult. Rapid progression of autophagy leads to cell death. Breast cancer cells restored for NMI expression showed autophagic vacuoles and LC3 processing. We found that NMI prompts activation of GSK3-β, a key kinase upstream of the TSC1/TSC2 complex. Inhibition of GSK3-β in NMI expressing cells activated mTOR signaling and decreased the cells' autophagic response. Abrogation of NMI expression rendered cells resistant to cisplatin and doxorubicin. Our TCGA analysis revealed concurrent expression of NMI and DNA-damage regulated autophagy modulator 1 (DRAM1) in breast cancer specimens. Detailed molecular studies revealed that NMI sensitizes breast cancer cells to cisplatin through DRAM1 dependent autophagy.

Our findings elicit some interesting possibilities about NMI, regulation of EMT, and autophagy. It seems likely that induction of autophagy via NMI may be a vital element in EMT determination given that major drivers of EMT are susceptible to autophagic degradation. Importantly our observations imply that NMI and DRAM1 levels may offer key parameters towards selection of target patients for successful cisplatin treatment. Overall, our studies highlight the importance of autophagy in breast cancer treatment.

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Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic IV

Pre-Metastatic Niche

Chairperson: David Lyden, Weill Cornell Medical College

Tuesday September 29th

ECM Remodeling During Cancer Progression

Janine Erler,
Biotech Research & Innovation Centre, University of Copenhagen, Denmark

Metastasis is responsible for over 90% of cancer patient deaths. It is a complex, multi-step process strongly influenced by the microenvironment. One major driving force is extracellular matrix (ECM) remodelling that increases tissue stiffness enhancing tumour progression. We have shown that lysyl oxidase (LOX) plays a critical role in mediating ECM remodelling at primary and metastatic sites. LOX is a secreted amine oxidase that catalyses the cross-linking of collagens and elastin in the ECM. The physical properties of tissues are altered through LOX activity to enhance cell proliferation, adhesion, migration, invasion and angiogenesis. We have been particularly interested in how LOX prepares pre-metastatic tissues for the arrival of metastasising cancer cells.

More recently, we have been studying how hypoxia regulates cancer-associated fibroblasts to alter ECM remodelling. Cancer-associated fibroblasts (CAFs) interact with tumour cells and promote growth and metastasis. We have shown that CAF activation is reversible: chronic hypoxia deactivates CAFs, resulting in the loss of contractile force, reduced remodelling of the surrounding extracellular matrix and, ultimately, impaired CAF-mediated cancer cell invasion. Hypoxia inhibits prolyl hydroxylase domain protein 2 (PHD2), leading to hypoxia-inducible factor (HIF)-1a stabilisation, reduced expression of aSMA and periostin, and reduced myosin II activity. Loss of PHD2 in CAFs phenocopies the effects of hypoxia, which can be prevented by simultaneous depletion of HIF-1a. Treatment with the PHD inhibitor DMOG in an orthotopic breast cancer model significantly decreases spontaneous metastases to the lungs and liver, associated with decreased tumour stiffness and fibroblast activation. PHD2 depletion in CAFs co-injected with tumour cells similarly prevents CAF-induced metastasis to lungs and liver. Our data argue that reversion of CAFs towards a less active state is possible and could have important clinical implications.

Overall, our findings suggest that targeting ECM remodelling can be used to disrupt cancer progression.

How Lymphatic Endothelial Cells Help Transform the Draining Lymph Node Into a Pre-Metastatic Niche

Melody A. Swartz,
Institute for Molecular Engineering and Ben May Department of Cancer Research, University of Chicago, US

Lymphatic vessels and cancer metastasis have long been correlated: the presence of the lymphatic growth factors VEGF-C/D in the tumor microenvironment associates with increased metastasis and poor prognosis, and function-blocking antibodies against their main receptor VEGFR-3, which prevent new lymphatic growth, are being tested in clinical trials to prevent metastasis. On the other hand, VEGF-C likely plays complex roles in the tumor microenvironment; in addition to driving lymphatic expansion locally and in the draining lymph node (LN), it has also been shown to increase lymphatic drainage, alter immune cell transport to the LN, and increase levels of the lymphoid chemokine CCL21, which attracts leukocytes, naïve T cells, and regulatory T cells, among others. VEGF-C can be secreted by tumor-associated macrophages and even tumor cells themselves. In this study, we asked how VEGF-C activation of lymphatic endothelial cells (LECs) in the tumor microenvironment promotes metastasis. First, we found that VEGF-C dramatically alters the tumor stroma, both biomechanically (in terms of fibroblast activation, matrix remodeling, and stiffness) as well as immunologically, particularly in its support of immune cell infiltrates and suppressive cytokine environment. Second, VEGF-C promotes tumor cell invasion into lymphatic vessels by hijacking mechanisms used for enhanced leukocyte infiltration during inflammation. Third, we found that LECs actively scavenge antigens and can process and present them on MHCI and MHCII molecules for direct T cell education, which promote dysfunctional (non-effector) activation in the presence of high levels of PD-L1 on LECs; this in turn can dampen anti-tumor T cell responses. And finally, we find that lymph draining the tumor is rich in tumor antigens and exosomes, which in turn are likely to transform the lymph node into an immune tolerant environment. Together, these data suggest that VEGF-C/VEGFR-3 targeting could potentially be important not only for preventing lymphangiogenesis, but also in strategies that aim to alter the tumor microenvironment in order to make traditional therapies and immunotherapies more effective.

Continued on next page.

While the traditional view of lymphatic vessel function is to drain excess fluid from peripheral tissues and return them to the blood circulation, there is a growing appreciation for lymphatic endothelial cells (LECs) as important players in immunity, as they are the first cells that come into direct contact with peripheral antigens, cytokines, danger signals and immune cells travelling from peripheral tissues to lymph nodes. In the tumor microenvironment, we are beginning to understand their importance not only in shaping the immune microenvironment but also in communicating with the immune system via the sentinel or tumor-draining lymph node. They also form conduits in the lymph node that direct different molecules to different cells, for example to B cells and immature dendritic cells, in turn helping to regulate the spatial and temporal kinetics of antigen presentation.

Our lab aims to understand how lymphatic transport functions affect and regulate immunity, which has led to new discoveries of LEC immune functions. For example, we recently demonstrated that LECs can take up exogenous antigens and load them intracellularly onto MHC class I molecules (a process referred to as cross-presentation), and directly present them to naïve T cells, leading to immune tolerance. The extent to which LECs scavenge vs. transport antigens depends on danger signals and cytokines present locally, which they also sense. Furthermore, in contrast to passive drainage, which is driven by translymphatic pressure differences, we are now appreciating the degree to which LECs can actively transport fluid and molecules by nonspecific vesicles. By directly controlling local lymphatic drainage rates, LECs not only regulate the kinetics of antigen transport to the lymph node but also modulate local interstitial flow. This interstitial flow can, in turn, help direct dendritic cells to the lymphatic vessel by virtue of a phenomenon we have termed autologous chemotaxis, whereby DCs express both lymphoid chemokines and their receptor and upon secretion, local gradients form by interstitial flow, chemotactically directing the DC to the lymphatic vessel. Thus, lymphatic vessels help shape immune responses through both physical and molecular mechanisms that are inherently coupled. We believe that integrative studies of lymphatic transport physiology with immunobiology is critical in revealing and understanding the key roles that lymphatic vessels play in cancer progression and metastasis as well as chronic inflammation and autoimmunity.

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METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic V

Disseminated, Dormant and Metastasis-Initiating Tumor Cells

Chairperson: David Lyden, Weill Cornell Medical College

Tuesday September 29th

The Origins of Disseminated Cancer Cells and Their Role in Dormancy and Metastatic Reactivation

Julio Aguirre-Ghiso,
Tisch Cancer Institute, Black Family Stem Cell Institute, Ichan School of Medicine at Mount Sinai, New York, US

The mechanisms that produce early-disseminated cancer cells (eDCC) during microscopically pre-invasive stages of breast cancer are poorly understood. Here we show that HER2+ mammary epithelial cells during atypical ductal hyperplasia (ADH) stages, are highly efficient in systemically disseminating from primary sites. These eDCCs were $HER2^{+}$/P-$ATF2^{lo}$/E-$cadherin^{lo}$, and were found also in human DCIS samples. Further, eDCC precursors in ADH lesions underwent a Wnt-dependent EMT that was reversed by HER2 or Wnt signaling blockade. Intra-vital imaging of transgenic HER2-CFP ADH lesions revealed that eDCC precursors invade the local stroma, intravasate and circulate to target organs. This process was aided by macrophages in similar way as they regulate mammary branching morphogenesis. We found that macrophages are localized inside the epithelium of pre-malignant lesions but localize in the stroma of healthy tissue. Macrophage recruitment depends on upregulation of CCL2 in HER2+ mammary epithelial cells *via* activation of NFkB. Importantly, samples from patients with DCIS frequently contain intra-epithelial macrophages, correlating with E-Cadherin downregulation. Macrophage invasion into ducts disrupts the myoepithelium and further supports the oncogene-driven EMT in luminal cells and their spread. Surprisingly, although the vast majority of eDCCs are non-proliferative, they can still initiate metastasis. However, depletion of macrophages from pre-malignant HER2+ lesions drastically reduces early dissemination and late metastasis. We reveal that during a stage where cancer is considered benign, HER2 signaling aberrantly activates a side-branching morphogenetic program that through macrophage recruitment causes early dissemination. That eDCCs carry latent metastatic initiating capacity cancer changes our understanding of metastasis onset and how it might be targeted effectively.

Where the Wild Things Are: The Perivascular Niche Regulates Disseminated Tumor Cell Dormancy and Drug Resistance

Cyrus Ghajar,
Fred Hutchinson Cancer Research Center, Seattle, USA

In a significant fraction of breast cancer patients, distant metastases emerge after years or even decades of latency. How disseminated tumor cells (DTCs) are kept dormant, and what wakes them up, are fundamental problems in tumor biology. To address these questions, we use metastasis assays in mice and zebrafish and have determined that the perivascular niche of distant sites like the lung, bone marrow and brain regulate DTC dormancy. We have developed organotypic microvascular niches to specify that endothelial cells regulate breast cancer cell growth, and applied proteomics to identify endothelial-derived mediators of DTC dormancy. More recently, we have begun to explore whether the perivascular niche confers therapeutic resistance to DTCs. I will present data that suggests strongly that the perivascular niche regulates therapeutic resistance of DTCs in a manner that is independent from its role in regulating DTC growth. Our goal is to uncover these mechanisms to guide strategies to eradicate dormant DTCs.

The Natural History of Metastasis: Why Does Adjuvant Chemotherapy Work?

Ben Z. Stanger,
University of Pennsylvania, Philadelphia, US

Despite the importance of metastasis, many of the steps involved in metastatic progression remain poorly characterized. Likewise, it remains unknown whether the size, microenvironment, or other features of a metastatic lesion dictates the efficacy of chemotherapy in the adjuvant (micro-metastatic) setting. To better characterize the natural history of metastasis, we used lineage tracing to study an autochthonous model of pancreatic ductal adenocarcinoma (PDAC), examining the evolution of disseminated cancer cells and the associated microenvironment during metastatic progression. PDAC tumors primarily metastasized to the liver, where small lesions seeded close to portal veins and exhibited a mesenchymal phenotype. With increasing size, lesions exhibited a greater epithelial phenotype, lower vessel density, and accumulation of a desmoplastic stroma, largely recapitulating the composition of the primary tumors from which they arose. Moreover, treatment with gemcitabine and nab-paclitaxel (a standard-of-care chemotherapy combination for patients with metastatic PDAC) significantly reduced the overall number of metastases and shifted the size distribution of metastases toward small lesions. These results provide an unprecedented look at metastatic progression – from single cells to macroscopic lesions – and suggest that adjuvant chemotherapy affords a survival benefit by directly targeting small metastases.

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METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic VI

Organ-Specific Metastasis and Micrometastatic Disease

Chairperson: David Lyden, Weill Cornell Medical College

Tuesday September 29th

Metastasis Initiating Cells, Niches and Vital Pathways

Joan Massagué,
Memorial Sloan Kettering Cancer Center, New York, US

Brain metastases occur in 20-40% of advanced stage cancers and represent the most prevalent intracranial malignancy in adults. Despite treatment advances at other metastatic sites, current clinical management of brain metastases affords limited disease control and most patients succumb to tumor progression less than twelve months after diagnosis. Focusing on brain metastasis from breast and lung cancers we found that circulating cancer cells traverse the blood-brain barrier (BBB) aided by a number of cancer cell-autonomous mediators of extravasation (EGFR ligands, prostaglandins, extracellular proteases, and sialyltransferases). Within the brain parenchyma, the infiltrating cancer cells die massively from exposure to astrocytes. Reactive astrocytes generate plasmin, which mobilizes the pro-apoptotic cytokine FasL to kill the infiltrating cancer cells. Brain metastatic cells can evade this attack by expressing serpins (neuroserpin, serpin B2) that inhibit plasminogen activator. The surviving cancer cells spread over brain capillaries by means of the neural cell adhesion molecule L1CAM. Metastatic cells proliferate on the coopted microcapillary basal lamina, forming a sheath that engulfs the local capillary network and eventually grows into a macrometastatic tumor. When devoid of L1CAM, circulating cancer cells can still infiltrate tissue and survive in the short term, but fail to coopt the vasculature and to grow. Astrocytes however are not uniformly antagonistic to invading cancer cells. Growing metastatic lesions contain abundant astrocytes, and astrocytes can have protective effects on cancer cell co-cultures *in vitro*. We identified the neural member of the cadherin family protocadherin 7 (PCDH7) as a component that brain metastatic cells employ to selectively engage astrocytes in the formation of protective connexin-43 gap junctions. Pharmacologic modulators of gap junctions and conditional inhibitors of L1CAM are under investigation as treatments of established brain metastasis in our model systems.

Authors: Joan Massagué, Qing Chen, Adrienne Boire, Manuel Valiente, Emrah Er, and Karuna Ganesh
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Tumor-Derived Vesicles Initiate Organtropic Niches

David Lyden,
Weill Cornell Medical College, New York, US

Metastasis to distant vital organs such as lung, liver, and brain is the most devastating feature of cancer progression, responsible for over 90% of cancer-associated deaths. In 1889, Stephen Paget first proposed that organ distribution of metastases is a non-random event, yet metastatic organotropism remains one of the greatest mysteries in cancer biology. Our recent studies uncovered that tumor-derived microvesicles, specifically exosomes, alter the microenvironment at future sites of metastasis to form pre-metastatic niches, creating a favorable "soil" for incoming metastatic "seeds". However, by what mechanism this occurs, and the role of exosomes in tumor metastasis, remains unknown. To investigate the role of exosomes in organotropic metastasis, we have used two established organotropic human tumor models: the MDA-231 breast cancer (BC) cell line, and its variants known to metastasize to the lung, brain and bone, respectively, as well as two liver metastatic pancreatic cancer (PC) cell lines, BxPC3 and HPAF-2. We first analyzed the biodistribution of fluorescently-labeled exosomes derived from lung metastatic, brain metastatic or bone metastatic MDA-231 BC variants or PC cell lines, and found that BC exosomes follow the organ-specific distribution of the cells of origin, while PC exosomes home to the liver. In each target organ exosomes are taken up by different cell types: fibroblasts/epithelial cells in the lung, Kupffer cells in the liver, and endothelial cells in the brain. In the organotropic MDA-231 model, prior education with the lung tropic exosomes redirected metastasis of the bone tropic cells to the lung, demonstrating the unique capacity of exosomes to determine the site of metastasis. Unbiased proteomic profiling of exosomes revealed distinctive protein expression patterns, and analysis of plasma exosomes from BC and PC patients that later developed site-specific metastasis revealed that exosome protein content could predict metastatic spread.

Authors: Ayuko Hoshino, Bruno Costa-Silva, Cyrus Ghajar, Irina Matei, Hector Peinado, Jacqueline Bromberg, David Lyden,
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METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic VII

Imaging Early Metastatic Events

Chairperson: Erik Sahai, London Research Institute, UK

Tuesday September 29th

Modelling Cancer Cell Invasion in Complex Environments

Erik Sahai,
London Research Institute, UK

My presentation will be split into two parts: the first will focus on cancer cell invasion and the second part will investigate how the microenvironment contributes to therapy failure.

Cancer cells can use different migratory strategies, particularly when challenged with complex three-dimensional matrices *in vivo*. This presents a particular problem when attempting to extrapolate findings from simple *in vitro* experiments to the complex matrix environments that surround tumors. To address this we have developed an agent based-finite element model of cell motility within different ECM topologies. Plasticity of migratory behavior is an emergent property of the model. We will present analysis of the underlying causes of this behavior together with testing of model predictions using conventional cell biology methods, intravital imaging of cancer cell migration, and metastasis assays.

Many tumors show an initial response to targeted therapies before genetic resistance emerges, however little is known about how tumor cells tolerate therapy before genetic resistance dominates. We present data that shows how the ECM generates a 'safe haven' in which melanoma cells can tolerate targeted therapy. This supports the population of cancer cells from which genetically resistance emerges. Finally, we present analysis of organ specific responses to targeted therapy in melanoma.

Regulation of Tumor Immunity by Extracellular Vesicles: Analysis in Context

Mikael Pittet,
Harvard University, Cambridge, US

There is compelling evidence that heterotypic interactions between neoplastic and seemingly normal host cells, including immune cells, profoundly regulate cancer progression. With this in mind, we are particularly interested in two aspects of cancer–host cell interplay. First, cancer cells interact with immune cells not only within the immediate tumor microenvironment but also in remote body locations. While the molecular pathways involved in local immune-neoplastic interactions have been intensively investigated, tumor influence on host systemic environments remains poorly understood. Second, an important communication mode between cancer cells and immune cells may involve tumor-derived extracellular vesicles (tEVs). Initial in vitro studies revealed that tEVs may engage, and therefore control, virtually any immune cell type. Further progress has been barred by our limited ability to understand the impact of tEVs produced in vivo. Therefore, we have combined new genetic approaches and molecular imaging to track endogenously produced tEVs and their targets at different resolutions and scales (organismal, cellular and molecular). We have evidence from in vivo studies that endogenous tEVs interact only with discrete immune cell subsets and that these interactions occur in restricted locations in the body. Consequently, remote interactions between cancer cells and host cells via tEVs likely control important aspects of cancer progression and are candidate targets for anti-tumor therapy.

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METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic VIII

Targeting Metastasis

Chairperson: Erik Sahai, London Research Institute

Tuesday September 29th

Targeting the HIF Pathway Therapeutically to Treat Metastasis

Amato J. Giaccia,
Stanford University School of Medicine, Stanford University, California, US

The receptor tyrosine kinase AXL has recently been identified as a critical factor driving tumor cell invasion and migration. AXL is the founding member of the TAM family of receptor tyrosine kinases, which include Tyro3 (or SKY), AXL, and MER. The ligand growth arrest specific gene-6 (GAS6) is the common ligand for all three receptors. We recently discovered that AXL is a hypoxia inducible gene and a HIF target gene. In addition, it is both a biomarker and genetic driver for several types of metastatic cancer. While AXL plays an important biologic role in metastasis, there are currently no therapeutic agents directed against GAS6/AXL signaling available that can be used to inhibit tumor progression and/or metastasis in clinical trials. We have developed a novel screening strategy to identify inhibitors of the GAS/AXL signaling axis. This strategy takes advantage of the fact that unlike other proto-oncogenic receptor tyrosine kinases (RTKs), AXL appears to be a suitable target for inhibition through modulation of ligand binding, given the lack of compelling data for ligand independent activation in cancer. We have engineered and produced wild type and ultra-high affinity soluble AXL FC-fusion proteins. In our studies, we demonstrate increased thermostability, affinity for GAS6, and therapeutic efficacy *in vivo* of the engineered AXL-S-1 soluble receptor compared to the wild type soluble AXL receptor. Furthermore, soluble AXL therapy has an added benefit over AXL monoclonal antibodies in being able to block GAS6 activation of the AXL family receptor tyrosine kinases MER and TYRO3, and can be engineered to increase its affinity and stability.

Authors: Mihalis S. Kariolis[1], Yu Rebecca Miao[1], Shannon Nash[1], Dadi Jiang[1], Anh Diep[1], Douglas S. Jones II[2], Shiven Kapur[2], Irimpan I. Mathews[3], Albert C. Koong[1], Erinn B. Rankin[1], Jennifer R. Cochran[2], Amato J. Giaccia[1]

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Identification and Therapeutical Targeting of Metastasis-Initiating-Cells in Human Oral Squamous Cell Carcinoma

Salvador Aznar Benitah,
Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain

Oral squamous cell carcinoma (SCC) is the 4th-5th most prevalent tumor in industrialized countries. It has a dismal prognosis, and severely affects the quality of life of patients. Resection of the tumor often requires aggressive surgery that removes large portions of the oral cavity including the tongue, fragments of the jaw, and inner oral mucosa. This not only leaves the patients severely scarred, but also significantly impairs their ability of feeding and talking. Approximately 50% of the patients develop lymph-node metastasis alone or in combination with lung metastasis. The prognosis of these patients is dismal with a 5-year survival below 20%. Recent studies indicate that the incidence of oral SCC is sharply rising in industrialized countries due to an increase in the number of infections with papillomavirus; based on these studies it is estimated that oral SCC will be the third most prevalent cancer within the next 30 years. My laboratory has developed a novel orthotopic model of patient-derived oral SCC that faithfully recapitulates the process of tumor growth and progression, including the development of spontaneous lymph node and lung metastases. This model is allowing us to monitor and study the process of metastatic spread in an unprecedented detailed manner. Using this system we have identified a population of cells within the primary tumor that constitutes the cell of origin of metastasis (i.e. metastasis-initiating-cells). These cells are defined by a signature that allows them to survive and thrive in the lymph nodes and bronchoalveolar environments. Interestingly, this signature indicates that metastasis-initiating-cells use a distinct type of metabolism that is adapted to take advantage of the specific nutrient environment present in the lymph nodes. Importantly, inhibition of this pathway either by shRNA or through pharmacological means completely prevents metastatic spread of patient-derived SCC tumors. Further underscoring its importance, activation of this pathway in non-metastatic tumors fully converts them into highly metastatic lesions. Analysis of public data indicates that the presence of this signature strongly correlates with a very poor prognosis in patients with oral SCC, but also in those with lung SCC, bladder cancer, ovarian cancer, or glioblastoma. I will discuss the potential therapeutic and prognostic value of our findings.

SHORT TALK

Cancer: Opening LOX to Metastasis

Thomas R. Cox

Biotech Research and Innovation Centre (BRIC), University of Copenhagen (UCPH), Copenhagen, Denmark

The metastasis of solid tumours remains one of the highest causes of mortality for cancer patients. Understanding the mechanisms by which primary tumours are able to colonise distant secondary organs to generate overt metastases remains a key challenge in cancer research.

The dynamic and reciprocal interaction between tumour cells and their microenvironment is crucial to many stages of development and progression. During progression and metastasis, the tumour extracellular matrix (ECM) is continuously remodelled both biochemically and biomechanically, feeding back onto tumour cells to enhance progression. Furthermore, the ability of tumour cells to alter ECM remodelling and re-program host cells at distant pre-metastatic sites prior to their arrival is a chilling reality.

We have shown that Lysyl Oxidase (LOX), a secreted ECM remodelling enzyme is critical to both breast and colorectal cancer progression. LOX expression at primary tumours leads to changes in ECM biochemistry and biomechanics, altering Src, FAK and NFATc1 signalling to drive progression and metastasis across multiple models. LOX activity modulates tissue stiffness leading to enhanced tumour growth, angiogenesis and metastasis. LOX is also crucially involved in pre-metastatic niche formation in multiple tissues including the lung, liver and bone, through altering recruitment and activation of host cells. The mechanisms contributing to primary tumour development, metastatic progression and tissue fibrosis share many commonalities. We have also shown that LOX is critical to the onset and development of tissue fibrosis and that LOX activity is key to establishing a milieu within fibrosing tissues that is favourable to colonisation and growth of metastatic tumour cells.

Together our data suggest that targeting LOX is a highly attractive target for blocking the pro-tumourigenic and pro-metastatic changes in the ECM during cancer progression and metastasis across multiple solid tumours.

SHORT TALK

LIPG Endothelial Lipase is Essential for Breast Cancer Growth and Metastasis Through Control of Lipid Supplies

Felipe Slebe[1], Federico Rojo[2,3], Maria Vinaixa[4,5,6] , Mar García-Rocha[1], Giorgia Testoni[1], Marc Guiu[1], Evarist Planet[1], Sara Samino[4,6], Antoni Beltran[4,6], Ana Rovira[2,7], Ana Lluch[8], Xavier Salvatella[1,11], Oscar Yanes[4,6], Joan Albanell[2,7,9], Joan J. Guinovart[1,6,10] and **Roger R. Gomis[1,11]**

[1] Institute for Research in Biomedicine (IRB Barcelona)
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[3] Pathology Department, IIS-Fundación Jimenez Diaz
[4] Centre for Omic Sciences, Universitat Rovira i Virgili
[5] Department of Electronic Engineering, Universitat Rovira i Virgili
[6] Spanish Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM)
[7] Medical Oncology Service, Hospital del Mar
[8] Hospital Clinico, Valencia
[9] Universitat Pompeu Fabra
[10] Universitat de Barcelona
[11] Institució Catalana de Recerca i Estudis Avançats (ICREA)

Cancer cells undergo metabolic reprogramming in order to produce biosynthetic precursors, such as adenosine triphosphate (ATP), lipids, nucleotides, and amino acids, to sustain the energy and substrate demands for rapid growth and proliferation. Among the various metabolic deregulations in cancer, lipid metabolism emerges as a critical pathway to maintain cell survival, growth, and migration. Thus research devoted to understanding the mechanisms of lipid metabolism adaptation in BC is highly relevant if we are to devise novel strategies to improve the control of this disease.

The mechanisms that allow breast cancer (BC) cells to metabolically sustain rapid growth are poorly understood. We report that BC cells are dependent on a mechanism to supply precursors for intracellular lipid production derived from extracellular sources and that endothelial lipase (LIPG) fulfills this function. Therefore, LIPG stands out as an essential component of the lipid metabolic adaptations that breast cancer cells, and not normal tissue, must undergo to support high proliferation rates. LIPG, is ubiquitously and highly expressed in all breast cancer subtypes but not in normal breast epithelial cells, and its down-regulation in transformed cells results in decreased proliferation, migration and impaired synthesis of intracellular lipids and an accumulation of extracellular lipids. LIPG expression is under the control of FoxA1 and FoxA2. These factors contribute to BC proliferation trough LIPG expression, thereby allowing extracellular generation and import of lipid precursors.

SHORT TALK

Endothelial Selectin-Mediated Recruitment of Myeloid Cells is Required for Tumor Cell Extravasation and Metastasis

I. Häuselmann, M. Roblek, D. Protsyuk, C. Stefanescu, **Lubor Borsig**

Institute of Physiology, Zürich Center for Integrative Human Physiology, University of Zürich, Switzerland

Metastasis is the primary cause of cancer-related mortality and the adopted metastatic microenvironment significantly contributes this process. Direct interactions between cancer cells and their microenvironment affect multiple steps during metastasis, particularly tumor cell seeding in distant organs and extravasation. Selectins are vascular receptors that facilitate tumor cell interactions with platelets, leukocytes and the endothelium. Activated endothelium at pre-metastatic niche is associated with the E-selectin expression. Here we provide evidence that E-selectin contributes to the recruitment of myeloid cells and enables tumor cell extravasation. Increased chemokine levels were detected in metastatic organs, which contributed to the initiation of the endothelial activation. Mechanistically ligation of E-selectin was required for an efficient endothelial retraction regulated by VE-cadherin phosphorylation. The absence of E-selectin attenuated spontaneous metastasis in several mouse models. Next we tested the hypothesis that local inhibition of chemokine CCL2 at metastatic sites can interfere with tumor cell extravasation and thereby reduce metastasis. The use of two different targeting strategies showed an effective inhibition of metastatic seeding and thereby metastasis. Taken together, we present a new mechanism how E-selectin promotes early metastatic niche formation and thereby cancer progression.

Molecular Regulation of the Critical Steps to Initiate Brain Metastasis

SHORT TALK

Manuel Valiente[1], Anna C. Obenauf[2], Xin Jin[2], Qing Chen[2], Xiang H.-F. Zhang[3], Derek J. Lee[2], Jamie E. Chaft[2], Mark G. Kris[2], Jason T. Huse[2], Edi Brogi[2], and Joan Massagué[2]

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Brain metastases constitute the most common neurological complication of cancer. We have recently found and characterized two critical steps for brain colonization that apply to seven different brain metastases models and a large number of human samples. Besides the important role of the blood-brain barrier to keep cancer cells out of the Central Nervous System, those that initiate metastatic lesions are equipped with additional components required to achieve the fitness to develop into macrometastases. Dissection of their role in brain metastases allowed us to uncover the molecular regulation of previously recognized hallmarks of secondary brain tumors: the high inefficiency of the brain metastatic phenotype and the absolute requirement of vascular co-option to initiate metastatic colonization. The presence of a strong response of the brain microenvironment to the presence of cancer cells has been broadly documented. We described how reactive astrocytes efficiently limit the number of metastatic clones that are initially seeding the brain parenchyma blocking their progression. However Neuroserpin and SerpinB2 provide metastatic cells the ability to avoid the response originated in the reactive microenvironment and resume colonization. In order to re-initiate proliferation in secondary organs we identify a cell adhesion molecule present in brain metastasis initiating cells that allows wrapping pre-existing capillaries in a process termed vascular co-option. L1CAM was functionally validated as a key driver of the vascular co-option program that ultimately makes possible to proliferate and grow in the perivascular niche. Given that many patients affecting with brain metastases have extra cranial secondary tumors as well, it is mandatory to prioritize novel targets that could potentially support viability in multi-organ metastases. Our more recent data provides this proof of concept for L1CAM.

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METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Topic IX

Modeling Metastasis

Chairperson: Cyrus Ghajar, Fred Hutchinson Cancer Research Center

Wednesday September 30th

Cooperative Events in Metastasis Identified Through Zebrafish Models

Richard Mark White,
Memorial Sloan Kettering Cancer Center, New York, US

Metastasis from melanoma is inevitably a collaboration between the tumor cells and the microenvironment, making in vivo models of the process essential to uncovering its mechanistic basis. In recent years, the zebrafish has emerged as an important cancer model due to its ease of genetic manipulation combined with the imaging capabilities afforded by the transparent casper strain. We have developed a zebrafish model of melanoma in which the human BRAFV600E gene is expressed under the melanocyte-specific mitf promoter. When crossed with p53-/- animals, the resultant BRAF;p53 fish develop a 100% penetrant melanoma. From these animals, we derived a series of fluorescent cell lines which can be transplanted into casper and allow for real-time imaging of metastatic dissemination at the single cell level. Using quantitative imaging, we have observed that successful macrometastases undergo a re-differentiation program, in which they simultaneously express high levels of SOX10/EDNRB along with the differentiation markers PMEL/TYRP1. This "semi-differentiated" state can be promoted by enforced expression of SNAI2, which promotes the expression of differentiation markers at secondary sites. Using competitive transplantation of GFP/RFP cells, we find that the semi-differentiated cells have a 5-fold advantage at secondary sites when compared to the parental cells. This advantage only occurs in vivo, suggesting that factors from the microenvironment support this state. To identify those factors, we have used RNA-seq to profile the metastatic cells, and find a significant enrichment of genes involved in dopamine and endothelin signaling, along with significant alterations in lipid metabolism. These factors play divergent roles in metastatic colonization, with some favoring differentiation while others favor proliferation, but together cooperate to enable successful colonization. Together, our data suggests that seemingly divergent microenvironmental factors can cooperate to produce the semi-differentiated cells that are the most capable of macrometastatic colonization.

Understanding Metastatic Predisposition Through Genome-Wide Screens for Metastasis Susceptibility Genes

Kent W. Hunter,
Center for Cancer Research, National Cancer Institute, Bethesda, US

Metastasis is a complex process involving many different molecular mechanisms and cellular systems and is the primary cause of patient morbidity and mortality for most solid tumors. Recent advances in genomic sequencing technologies and informatics have provided major insights into the mutational spectrum and etiology of solid tumors. This information is an important foundation upon which much of the current efforts in precision medicine are based, which aims to tailor individual patient therapy based on personal oncogenic mutational load. In contrast, despite the critical role in patient mortality, significantly less is known about factors that contribute to metastasis. Greater depth of knowledge regarding the metastatic process would likely provide significant advances in patient care by directly targeting the molecular mechanisms that establish and support the growth of these life-threatening lesions. Here we report the results of a integrated genetic, transcriptional, and epigenetic analysis in a mouse mammary tumor model to identify factors associated with tumor progression on a genome-wide scale. This strategy identified novel metastasis susceptibility genes and a transcription signature that is prognostic for late metastatic disease. The analysis implicates a number of upstream transcriptional regulators for metastatic disease and suggests cell-mediated immunity is an important determinant of metastasis. Furthermore, this analysis re-identified current standards of care for adjuvant therapy and identified novel or other FDA-approved drugs as potentially useful for anti-metastatic therapy. Finally, this integrated strategy enabled the development of panels of single nucleotide polymorphisms (SNPs) that predict breast cancer survival from constitutional DNA, suggesting a blood-based assay might be developed for breast cancer prognosis. Further explorations implementing this strategy may therefore provide a variety of information for clinical applications in the control and treatment of advanced neoplastic disease.

Whole-Body Analysis of Premetastatic Niche Formation Identifies MIDKINE as a Novel Driver of Lymphangiogenesis and Metastasis in Melanoma

María S. Soengas,
Spanish National Research Cancer Centre, Madrid, Spain

Melanoma is the only cancer type where seemingly thin cutaneous lesions (2 mm in depth) can be at risk for dissemination to sentinel lymph nodes, and ultimately, to visceral sites. Still, how lymphatic remodeling contributes to distal metastasis, tumor relapse and drug response remains unclear. This is mainly due to the lack of markers and experimental models for non-invasive imaging of lymphangiogenesis in vivo. Here we present a Vegfr3-luciferase knock-in strategy engineered to generate immunocompetent and immunodeficient reporter mice for spatio-temporal analyses of melanoma-driven lymphangiogenesis. This strategy revealed systemic multifocal neo-lymphangiogenesis as an early event in melanoma development, defining metastatic potential and marking metastatic relapse after surgery. Analysis of the melanoma secretome revealed MIDKINE (MDK) as a novel driver of these pre-metastatic niches, with unanticipated roles both on lymphatic and tumor cell compartments. The physiological relevance of MDK was demonstrated in long-term follow up analyses of disease-free patient survival. Additional data will also be discussed in the context novel anti-lymphangiogeneic factors identified and validated in the Vegfr3-lymphoreporter mice, further expanding the versatility of these unique animal models.

David Olmeda[1], Daniela Cerezo-Wallis[1], Erica Riveiro-Falkenbach[2], Nuria Ibarz[3], Lisa Osterloh[1], Tonantzin G. Calvo[1], Francisca Mulero[4], Diego Megías[5], Javier Muñoz[3], Pablo Ortiz-Romero[2], José L Rodriguez-Peralto[2], Sagrario Ortega[6] and María S. Soengas[1]

[1] Melanoma Laboratory, Molecular Oncology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[2] Instituto de Investigación i+12, Hospital 12 de Octubre, Universidad Complutense Madrid Medical School, Spain
[3] Proteomics Unit, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[4] Molecular Imaging Unit, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[5] Confocal Microscopy Unit, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[6] Transgenic Mice Unit Biotechnology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

SHORT TALK

Astrogliosis and Neuroinflammation are Instigated in a Mouse Model of Spontaneous Melanoma Brain Metastasis

Hila Schwartz, Eran Blacher, Malak Amer, Nir Livneh, Anat Klein, Dikla Ben-Shushan, Shelly Soffer, Raquel Blazquez, Alonso Barrantes-Freer, Meike Müller, Karin Müller-Decker, Ronit Satchi-Fainaro, Viktor Umansky, Tobias Pukrop and **Neta Erez**

Tel Aviv University, Tel Aviv, Israel
University Hospital Regensburg, Regensburg, Germany
University Medical Center Göttingen, Göttingen, Germany
German Cancer Research Center, Heidelberg, Germany
University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany

Malignant melanoma is the deadliest of all skin cancers. Melanoma frequently metastasizes to the brain, resulting in dismal survival. One of the major obstacles for characterizing mechanisms of brain metastasis is the lack of tractable pre-clinical models. We established a mouse model of spontaneous melanoma brain metastasis that gives rise to both micro- and macro- metastases in immunocompetent mice, that can be utilized to study the early stages of metastases formation, as well as the reciprocal interactions of brain metastasizing melanoma cells with the brain microenvironment. Moreover, we developed tools for detection and quantification of brain micrometastases and for intravital diagnosis by analyzing blood and cerebrospinal fluid (CSF). These tools can be implemented in pre-clinical studies.

Utilizing this model, as well as a transplantable model with a patient-derived cell line, we show that melanoma brain metastases are associated with reactive astrogliosis and activation of neuroinflammation and a woundhealing program in astrocytes. Our findings suggest that astrogliosis, physiologically instigated as a brain tissue damage response, is hijacked by tumor cells to support metastatic growth. Elucidating the molecular mechanisms that govern metastatic dormancy and early metastatic growth is the key to developing novel therapeutic approaches that may prevent brain metastatic relapse.

SHORT TALK

Tracking Rare and Dynamic Events *in vivo* at High Resolution Using Multimodal Correlative Microscopy

Jacky G. Goetz[1] and Yannick Schwab[2] and colleagues

[1] INSERM U1109, Strasbourg, France
[2] EMBL, Heidelberg, Germany

Three reasons explain why most of the critical events driving normal and pathological scenarios had been less investigated: they occur rarely in space and time, they are highly dynamic, they differ when studied in situ in an entire living organism. Metastasis is the primary cause for cancer-related mortality, but its mechanisms remain to be elucidated. Intravital imaging has opened the door to *in vivo* functional imaging in animal models of cancer, however it is limited in resolution. Ultrastructural analysis of tumor metastasis *in vivo* has so far been hindered by the limited field of view of the electron microscope, making it difficult to retrieve volumes of interest in complex tissues. We recently developed a multimodal correlative approach allowing us to rapidly and accurately combine functional in vivo imaging with high-resolution ultrastructural analysis of tumor cells in a relevant pathological context. The multimodal correlative approach that we propose here combines two-photon excitation microscopy (2PEM), microscopic X-ray computed tomography (microCT) and three-dimensional electron microscopy (3DEM). It enables a rapid and accurate correlation of functional imaging to high-resolution ultrastructural analysis of tumor cells in a relevant pathological context. As an example, we are now capable of providing highand isotropic (8nm) resolution imaging of single metastasizing tumor cells previously imaged in the process of extravasation in the living mouse brain. This reliable and versatile workflow offers access to ultrastructural details of metastatic cells with an unprecedented throughput opening to crucial and unparalleled insights into the mechanisms of tumor invasion, extravasation and metastasis *in vivo*.

SHORT TALK

Chemotherapy Alters the Natural History of Metastatic Progression

Nicole M. Aiello, David L. Bajor, Neha Bhagwat, Minh Pham, Toby C. Cornish, Christine A. Iacobuzio-Donahue, Robert H. Vonderheide, Ben Z. Stanger

Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, US
Department of Pathology, Johns Hopkins University, Baltimore, US
Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins School of Medicine, Baltimore, US
Rubenstein Center for Pancreatic Cancer Research, Memorial Sloan Kettering Cancer Center, New York, US

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a 5-year survival rate of less than 6%. Metastatic disease accounts for a majority of PDAC-related deaths, even for patients with no evidence of metastasis at the time of resection. In this study we sought to delineate the natural history of metastatic colonization and determine whether size, microenvironment or other features correlate with the response of metastatic lesions to chemotherapy. Using an autochthonous PDAC mouse model combined with a lineage-labeling approach, we have tracked and characterized the various stages of metastatic progression, from single cells to macroscopic lesions. PDAC tumors primarily metastasize to the liver, where small lesions reside closest to portal veins and exhibit a high frequency of epithelial-mesenchymal transition (EMT). Larger metastatic lesions are predominantly epithelial and hypovascular, resembling primary PDAC tumors. Metastases gradually accumulate desmoplasia as they grow, which consists of myofibroblasts, leukocytes and extracellular matrix components including collagen, hyaluronic acid, fibronectin and SPARC. Treatment with gemcitabine and nab-paclitaxel reduces overall metastatic burden and shifts the size distribution of metastases toward small lesions of 10 cells or less. Single disseminated cells in particular seem to be protected from chemotherapy-induced killing, while larger lesions that have accumulated a desmoplastic stroma are more susceptible. These results provide an unprecedented look at metastatic progression – from single cells to macroscopic lesions – and suggest that adjuvant chemotherapy affords a survival benefit by directly targeting micrometastases.

SHORT TALK

Genome-Wide *in vivo* Screening Identifies Novel Microenvironmental Regulators of Metastatic Colonisation

Louise van der Weyden[1], Anneliese O. Speak[1], Mark. J Arends[2], Sanger Mouse Genetics Project[1], and David J. Adams[1]

[1] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
[2] University of Edinburgh Division of Pathology, Edinburgh Cancer Research Centre, Institute of Genetics & Molecular Medicine, Western General Hospital, Edinburgh, UK

Metastasis is the leading cause of death for cancer patients. This multi-stage process requires tumour cells to survive in the circulation, extravasate at distant sites and grow, and requires contributions from both the tumour cell itself and the microenvironment. The ability of the microenvironment to regulate growth of the tumour cells after extravasation ('colonisation') is an important determinant of metastatic outcome. Here we show the results of screening 645 knockout mouse lines using an in vivo experimental metastasis assay to identify microenvironmental regulators of metastatic colonisation. We identified 18 genes that when disrupted in the mouse, modify the ability of intravenously administered tumour cells to establish metastatic foci in the lungs; 14 of these genes have not previously been shown to play a role in metastasis. The strongest reduction in pulmonary metastasis was observed in sphingosine-1-phosphate (S1P) transporter Spinster Homolog 2 (Spns2)-deficient mice. We demonstrate a novel bilateral mechanism whereby suppression of Spns2 creates a hostile microenvironment, through increased levels of pro-apoptotic ceramides and sphingosine in the lungs, and also increased activity of the immune system, by adjusting the balance between the regulatory and tumour killing effector T cell subsets, resulting in potent tumour cell killing and an overall decreased metastatic burden.

Madrid 28-30 September 2015

METASTASIS INITIATION — MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

Speakers' Biographies

CNIO Frontiers Meeting
Metastasis Initiation: Mechanistic Insights and Therapeutic Opportunities
September 28-30, 2015

Julio A. Aguirre-Gisho

Professor, Director of Head and Neck Cancer Basic Research,
Director of Solid Tumor and Metastasis Research.
Division of Hematology and Oncology, Department of Medicine,
Department of Otolaryngology, Department of Oncological
Sciences, Tisch Cancer Institute, Black Family Stem Cell
Institute, Ichan School of Medicine at Mount Sinai,
New York, US

BIOGRAPHY

Dr. Aguirre-Ghiso is a tenured Full Professor, Director of Solid Tumor and Metastasis Research at the Ichan School of Medicine at Mount Sinai in New York. He discovered that specific cues in the microenvironment regulate a balance between mitogenic vs. stress signaling pathways to induce cancer cell dormancy. Translating this biology to medicine he identified a "dormancy signature" enriched in dormant DTCs from patients asymptomatic for up to 18 years and that predicts for prolonged metastasis-free periods in different cancers. He is also developing into a clinical trial an epigenetic reprograming therapy to induce dormancy of cancer cells and works with tha pharmaceutical industry to develop novel therapeutic strategies to reprogram tumor cells into dormancy or kill dormant cells.

Gemma Alderton

Senior Editor, *Nature Reviews Cancer*, London, UK

BIOGRAPHY

Gemma Alderton received her PhD in Biochemistry from the University of Sussex, Brighton, UK where she researched DNA damage and cell cycle signalling pathways and their deregulation in human disease with Penny Jeggo. Having established an interest in cell cycle signalling she worked as a postdoc in John Diffley's lab at the Cancer Research UK Clare Hall laboratories, London, UK, investigating the control of the G1/S checkpoint signalling in mammalian cells. In October 2006 she moved to Nature Reviews Cancer to continue her broad interest in pathological cell signalling. She is currently a senior editor and has particular interests in metastasis, the tumour microenvironment, tumour immunology, metabolism and genomic instability.

Victoria Aranda

Senior Editor, *Nature Medicine*, New York, US

BIOGRAPHY

Victoria Aranda is a senior editor with Nature Medicine, where she handles research manuscripts on basic and applied cancer research. Before joining Nature Publishing Group, Victoria carried out research on Breast Cancer and Cell Biology at Cold Spring Harbor Laboratory. Victoria received her Ph.D. from the University of Navarra in Spain, where she studied how alterations in epithelial polarity and tissue architecture contribute to liver disease.

As the senior oncology editor, and the community leader for all cancer editors in NPG, Victoria keeps track of the latest developments in translational cancer research, from cancer genomics to drug development and everything in between, and works in close relationship with the research community to ensure that Nature Medicine continues to publish cutting-edge, high-quality science in a rapidly evolving field. Her passion for cancer science takes her around the world attending to conferences and visiting research centers, where she is glad to put her editorial experience to the service of prospective authors, and to advise young scientists about the daunting world of publishing.

Salvador Aznar Benitah

ICREA Research Professor, Foundation Botín Researcher,
Institute for Research in Biomedicine (IRB Barcelona),
Spain

BIOGRAPHY

Salvador Aznar Benitah obtained his Honours Degree in Molecular Biology and Biochemistry at McGill University (Montreal, Canada) in 1998, and his PhD studies in 2003 in Molecular Oncology at the Biomedical Research Institute in Madrid (Spain). In 2003 he moved to London as a postdoctoral fellow in the laboratory of Prof. Fiona Watt at the *London Research Institute* (Cancer Research UK). He then established his own lab at the *Center for Genomic Regulation* (CRG) in 2007 as a *Junior ICREA researcher*. In September 2012 Salvador became an *ICREA Research Professor*, and in 2013 he moved his laboratory to the *Institute for Biomedical Research* (IRB) in Barcelona. He has recently been awarded in 2014 the *Banc Sabadell Investigator Award*, an *ERC Starting Grant*, and a *Foundation Botín Grant*.

Dr. Benitah´s lab aims at identifying and characterizing the molecular mechanisms underlying the function of adult stem cells. The recent work of his lab has been focused in understanding how adult stem cells are spatiotemporally regulated, how they communicate with their local and systemic environment, and how stem cell malfunction contributes to tissue ageing and tumorigenesis.

Thomas Brabletz

Chair Experimental Medicine I,
Nikolaus-Fiebiger-Center for Molecular Medicine,
University Erlangen-Nuernberg, Germany

BIOGRAPHY

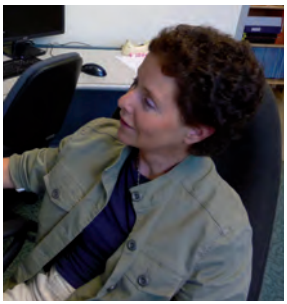
Thomas Brabletz is Professor for Molecular Oncology and Chair of the Dept. of Experimental Medicine, University Erlangen, Germany. Focusing on malignant cancer progression, in particular mechanisms of invasion and metastasis, he aims to integrate basic and clinically relevant cancer research. He proposed a concept of transient rounds of EMT and MET, resulting in aberrant cellular plasticity and the generation of 'Migrating cancer stem cells' as driving force of metastasis. Currently his major interests are in uncovering underlying mechanisms, such as feedback loops between EMT-inducers, oncogenic pathways and microRNAs, as basis for novel therapeutic strategies to fight cancer metastasis.

Janine Erler

Associate Professor, Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark

BIOGRAPHY

Dr. Janine Erler obtained a BSc(Hons) in Molecular Genetics at the University of Sussex in 2000. She then did a PhD in Molecular Pharmacology at the University of Manchester researching how tumor hypoxia drives chemoresistance, and graduated in 2003. After this, Janine went to Stanford University for four years for her post-doctoral training where she continued her research on hypoxia but now focused on how this drives metastasis. She then returned to the UK in 2008 to set up her own lab at the Institute of Cancer Research in London, and moved her lab to BRIC in Denmark in 2012.



Brunilde H. Felding-Habermann

Associate Professor, The Scripps Research Institute,
La Jolla, US

BIOGRAPHY

Studies in the Felding lab aim to understand mechanisms of tumor metastasis and to define new strategies for prevention and inhibition. A major focus is on breast cancer brain metastasis to unravel initial critical steps of tumor cell brain colonization, and to elucidate contributions of host vascular cells and the coagulation system. Approaches for interfering with cancer progression further seek to define new cancer specific targets and engineer antibodies for immune therapies and empowering of host immune responses. Recent studies focus on normalizing tumor cell metabolism to block cancer aggressiveness and prevent disease progression and recurrence.

Cyrus Ghajar

Assistant Member,
Public Health Sciences Dividion/Translational Research Program,
Human Biology Division, Hutchinson Cancer Research Center,
Seattle, US

BIOGRAPHY

Dr. Cyrus Ghajar directs the Laboratory for the Study of Metastatic Microenvironments (LSM2) (URL:http://research.fhcrc.org/ghajar/en.html) in Fred Hutchinson Cancer Research Center's Translational Research Program. Broadly, he is interested in how "foreign" tissue microenvironments influence the behavior of disseminated tumor cells (DTCs). Specifically, his laboratory is working to understand how tissues regulate DTC dormancy, how the dormant niche contributes to therapeutic resistance, and how local and systemic changes awaken DTCs. His ultimate interests lie in targeting dormant DTCs to prevent metastasis.

Amato Giaccia

Jack, Lulu & Sam Willson Professor in Cancer Biology.
Director, Division of Radiation & Cancer Biology,
Department of Radiation Oncology,
Stanford School of Medicine, US

BIOGRAPHY

Dr. Giaccia is a Professor of Radiation Oncology, Associate Chair for Research & Director of the Division of Radiation & Cancer Biology in the Department of Radiation Oncology. He also is the Director of Basic Science at the Stanford Cancer Institute and heads the Radiation Biology Program in Stanford's Cancer Center, and is Director of the Cancer Biology Interdisciplinary Graduate Program. He was awarded an American Cancer Society Junior Faculty Research Award and the Michael Fry Award from the Radiation Research Society for his outstanding contributions on understanding the molecular mechanisms of resistance promoted by the tumor microenvironment. Additionally, he was the recipient of the 2013 ASTRO Gold Medal. He co-authored the sixth & seventh editions of the textbook, "Radiation Biology for the Radiologist," with Professor Eric Hall from Columbia. In addition, he is currently the "Jack, Lulu and Sam Willson Professor in Cancer Biology" in the Stanford University School of Medicine. Dr. Giaccia was recently awarded an R35 grant from the NIH (Outstanding Investigator Award).

Kent W. Hunter

Senior Investigator, Head, Metastasis Susceptibility Section,
Laboratory of Cancer Biology and Genetics,
Center for Cancer Research, National Cancer Institute,
Bethesda, US

BIOGRAPHY

Dr. Hunter received a B.S. in biochemistry with Highest Honors from the Pennsylvania State University in 1985 and a Ph.D. in Biology from the Massachusetts Institute of Technology in 1991. He was an Associate Member at the Fox Chase Cancer Center from 1996 to 1999. In 1999, he joined the Laboratory of Population Genetics at the National Cancer Institute, National Institutes of Health in Bethesda, Maryland, USA as an Investigator and became a Senior Investigator in 2007. He currently is a member of the Laboratory of Cancer Biology and Genetics.

Joan Massagué

Director, Sloan Kettering Institute, Alfred P. Sloan Chair
Member, Cancer Biology Genetics Program
Director, Metastasis Research Center, Memorial Sloan-Kettering Cancer Center, New York
Professor, Weill Graduate School of Medicine, Cornell University, US

BIOGRAPHY

Joan Massagué (Barcelona, 1953) received his Ph.D. degree from the University of Barcelona in 1978. He joined the faculty at the University of Massachusetts Medical School in 1982. In 1989 he was appointed Alfred P. Sloan Chair in Cancer Biology, Memorial Sloan-Kettering Cancer Center, New York, and Howard Hughes Medical Institute Investigator. He assumed his current position as Director, Sloan-Kettering Institute, in 2014.

Dr. Massagué is a world leader in the signaling pathways and transcriptional programs that regulate normal cell behavior and cancer metastasis. He identified the TGFβ receptors, their mechanism of signal transduction, and the central concept of how this pathway controls cell division and developmental fate. He provided a direct explanation for how external signals block cell division through CDK inhibitors and cell fate through chromatin regulators. These mechanisms are crucial in embryonic development, and their disruption causes congenital disorders and cancer. Dr. Massagué recently identified genes and mechanisms of metastasis to bones, lungs and the brain, thus illuminating the basis for cancer lethality and opening new avenues for treatment.

Dr. Massagué is a member of the U.S. National Academy of Sciences, the Institute of Medicine, the American Academy of Arts and Sciences, the Spanish Royal Academies of Medicine and of Pharmacy, and the European Molecular Biology Organization. Noteworthy recognitions include the Passano Prize, the Vilcek Prize, the BBVA Frontiers of Science Prize, the Prince of Asturias Prize, the Pasarow Prize and the Charles Rodolphe Brupbacher Prize for Cancer Research.

Yibin Kang

Warner-Lambert/Parke-Davis Professor of Molecular Biology,
Molecular Biology, Princeton University,
Princeton, NJ, US

BIOGRAPHY

Yibin Kang is a Warner-Lambert/Parke-Davis Professor of Molecular Biology at Princeton University. A graduate of Fudan University, Dr. Kang completed his graduate study at Duke and postdoctoral training at the Memorial Sloan-Kettering Cancer Center. He joined the faculty of Princeton University in 2004 and was promoted to Associate Professor in 2010 and to Endowed Chair Professor in 2012. Dr. Kang is the President-Elect of the Metastasis Research Society. Dr. Kang's outstanding achievements in metastasis research have been recognized by many prestigious awards, including the 2011 Vicek Prize and the 2012 AACR Award for Outstanding Achievements in Cancer Research.

David Lyden

Stavros S. Niarchos Professor, Department of Pedriatics,
and Cell and Developmental Biology,
Weill Cornell Medical College, New York, US

BIOGRAPHY

Early work in Dr. Lyden's laboratory resulted in several fundamental discoveries that involve the role of bone marrow-derived stem and progenitor cells in tumor vasculogenesis and in metastasis. Dr. Lyden and colleagues subsequently identified two bone marrow-derived cell types, endothelial progenitor cells (EPCs) and hematopoietic progenitor cells (HPCs) of myeloid origin that both participate in the formation of new blood vessels in the primary tumor that occurred by vasculogenesis as opposed to angiogenesis. Dr. Lyden's laboratory then went on to show that secreted factors by the primary tumor prime certain tissues for tumor cell engraftment. His laboratory defined the concept of the "pre-metastatic niche". At the pre-metastatic niche, newly recruited bone marrow-derived myeloid progenitor cells collaborate with other cells types residing in the tissue parenchyma. Together, these cells provide a platform of pro-inflammatory molecules, such as S100 family members, growth factors, matrix-degrading enzymes (MMP9) and adhesion molecules (fibronectin and laminin), thereby accelerating assembly of the metastatic lesion. Dr. Lyden's team investigation of tumor-secreted factors that mediate the crosstalk between tumors and cells in the remote metastatic microenvironment has led to his recent discovery that tumor-secreted microvesicles, known as exosomes, initiate pre-metastatic niche formation by educating stromal cells and bone marrow progenitor cells, thus supporting a pro-metastatic microenvironment. His laboratory has identified key proteins and the presence of nucleic acids in exosomes that support thrombosis generation and pre-metastatic niche formation, including vascular leakiness, at organotropic sites.

Ángela Nieto

CSIC Research Professor,
Developmental Neurobiology Unit,
Neuroscience Institute of Alicante, Spain

BIOGRAPHY

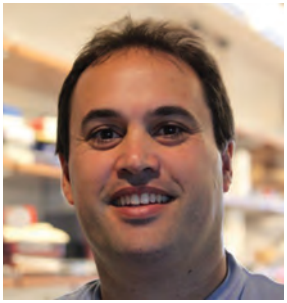
M. Angela Nieto is Full Professor and Head of Developmental Neurobiology at Instituto de Neurociencias (CSIC-UMH) in Alicante, Spain. PhD in 1987 (Universidad Autónoma, Madrid). Her main contribution has been the characterization of cell plasticity in health and disease, and in particular, the impact of reactivation of developmental programs in adult pathologies including cancer and organ degeneration. Elected member of EMBO (2000-) and Academia Europaea (2009-). She is the President of the Spanish Society for Developmental Biology, member of the Board of Directors International Society of Differentiation and the Spanish delegate at EMBL-EMBC and Vice-chair at EMBL Council.

Klaus Pantel

Professor of Medicine, Chairman, Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

BIOGRAPHY

Prof Pantel is Chairman of the Institute of Tumour Biology at the University Medical Center Hamburg-Eppendorf. The institute is part of the Centre of Experimental Medicine and the University Cancer Center Hamburg (UCCH). The pioneer work of Prof Pantel in the field of cancer micrometastasis, circulating tumor cells and circulating nucleic acids (ctDNA, microRNAs) is reflected by more than 350 publications in excellent high ranking biomedical and scientific journals (incl. NEJM, Lancet, Nature Journals, Cancer Cell, Science Translational Medicine, Cancer Discovery, PNAS, JCO, JNCI, Cancer Res.) and has been awarded the AACR Outstanding Investigator Award 2010, German Cancer Award 2010, and ERC Advanced Investigator Grant 2011. Moreover, Prof Pantel coordinates the European TRANSCAN group "CTC-SCAN", the European IMI consortium CANCER-ID (www.cancer-id.eu) on blood-based "Liquid Biopsies" and serves on the Editorial Boards of international cancer journals (e.g., Clin. Cancer Res., Breast Cancer Res., Cancer Res.).



Héctor Peinado Selgas

Head of the Microenvironment and Metastasis Group,
Molecular Oncology Programme,
Spanish National Cancer Research Centre, Madrid, Spain

BIOGRAPHY

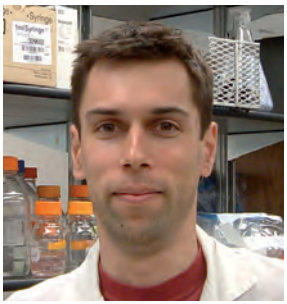
I did my PhD in the laboratory of Dr. Amparo Cano in Madrid (Spain, Biomedical Research Institute "Alberto Sols") where I specialized in analyzing Epithelial to Mesenchymal Transition Mechanisms. In this lab we described the molecular mechanisms of EMT regulated by Snail transcription factor and Lysyl Oxidase 2 (EMBO J (2005) 24:3446-58, Nat. Rev Cancer. (2007). 7:415-28). I defined a role for beta-catenin in regulating cancer stem cell behavior in skin cancer (Nature. (2008) 452:650-3). I joined Dr. Lyden's laboratory as a postdoctoral associate in 2008 to study the crosstalk between tumor cells and bone marrow derived cells during metastatic progression. During these years I developed my career as an independent researcher with an excellent collaboration with Dr. Lyden. In 2010, was appointed to a Faculty Position in the Department of Pediatrics at WCMC and in 2013 I was promoted to Assistant Professor. My work has recently defined that tumor-secreted exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype (Nature Medicine. 2012. (18): 883-891.). In 2015, I joined the CNIO as Group Leader of the Microenvironment and Metastasis laboratory with a Ramon y Cajal contract. My current research goals are focused on understanding the crosstalk between the tumor and its microenvironment by secreted factors during lymph node metastasis and determine new targets to block metastasis.

Mikael Pittet

Samana Cay MGH Research Scholar,
Associate Professor Harvard Medical School,
Center for Systems Biology, Massachusetts General Hospital,
Cambridge, US

BIOGRAPHY

Dr. Pittet completed his PhD at the Ludwig Institute for Cancer Research, Lausanne, Switzerland and trained as a postdoctoral fellow at Massachusetts General Hospital (MGH), Dana Farber Cancer Institute and Harvard Medical School (HMS), Boston, USA. He began his independent research in 2007 at the MGH Center for Systems Biology and currently serves as an Associate Professor at HMS. Work from the Pittet lab combines several approaches, including in vivo imaging, to uncover the maturational pathways, trafficking and functions of key immune cell types found in tumors and other tissues, with the goal of establishing new paradigms for translational efforts.



Erik Sahai

Head of the Tumour Cell Biology Laboratory,
The Francis Crick Institute, Cancer Research UK

BIOGRAPHY

Erik Sahai's research is focused on the spread of cancer through the body, how interactions between cancer cells and the tumour microenvironment affect this process and the response to cancer therapy. To study these problems his group uses a wide range of techniques from computational modelling of cell migration, through conventional cell and molecular biology, to intravital imaging of mouse tumours and live analysis of patient derived material. Erik obtained his PhD with Richard Treisman in London studying RhoGTPases and their effectors. He then carried out post-doctoral work in both London (Chris Marshall) and New York (John Condeelis) before setting up his own group at the Cancer Research UK London Research Institute in 2004.

Maria S. Soengas

Head of the Melanoma Group, Molecular Oncology Programme,
Spanish National Cancer Research Centre, Madrid, Spain

BIOGRAPHY

María S. Soengas is the Head of the Melanoma Group at CNIO. The long-term goal of her team is to translate basic research in melanoma into the clinic by identifying novel tumour markers and drug targets. In the context of tumor imaging, the Soengas group has engineered "metastasis-alert" animal models as a platform for gene discovery and pharmacological screens in vivo. In particular, they have identified innate immunity activators which are being exploited by a start up company she has cofounded (Bioncotech Therapeutics). Soengas has received multiple international awards and is actively involved in skin cancer prevention and awareness campaigns.

Ben Z. Stanger

Associate Professor of Medicine and Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, US

BIOGRAPHY

Dr. Stanger earned his SB in Life Sciences from MIT in 1988 and his MD/PhD from Harvard Medical School in 1997, where his graduate work concerned mechanisms of apoptosis. He then completed a residency in Internal Medicine at UCSF and a fellowship in Gastroenterology at the Massachusetts General Hospital, performing postdoctoral work on gastrointestinal development. In 2006, he moved to the University of Pennsylvania, where he is Associate Professor of Medicine and Cell and Developmental Biology. Dr. Stanger's research concerns the formation of gastrointestinal organs and how developmental programs are utilized during regeneration and carcinogenesis of these tissues.

Li-Kuo Su

Editor, *Cancer Cell*, Cambridge, US

BIOGRAPHY

Li-Kuo Su received his Ph.D. from the University of Pennsylvania where he did research on the transcriptional regulation of the murine immunoglobulin heavy chain locus with Tom Kadesh. He received postdoctoral training first at the Cold Spring Harbor Laboratory studying RB family proteins with Ed Harlow then at Johns Hopkins University studying the APC tumor suppressor with Bert Vogelstein. Li-Kuo joined the faculty of M. D. Anderson Cancer Center in 1995. In 2003, Li-Kuo moved to Cell Press to become the Editor of Cancer Cell.

Melody A. Swartz

Professor, Institute for Molecular Engineering and Ben May Department of Cancer Research,
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BIOGRAPHY

Melody A. Swartz is the William B. Ogden Professor in the Institute of Molecular Engineering at the University of Chicago, where she holds a joint appointment in the Ben May Department for Cancer Research. She obtained her BS from the Johns Hopkins University and her PhD from Massachusetts Institute of Technology, both in Chemical Engineering. She undertook postdoctoral studies at Harvard Medical School in Boston before joining Northwestern University as an Assistant Professor with joint appointments in the Departments of Biomedical Engineering and Chemical Engineering. In 2003 she moved to Switzerland as a founding member of the Institute of Bioengineering in the Ecole Polytechnique Fédérale de Lausanne (EPFL), where she later served as Department Chair, and also served a joint appointment in the Swiss Institute for Experimental Cancer Research. She moved to the University of Chicago in 2014 to be part of the new Institute, which represents the first engineering program in the University's history.

Trained as a bioengineer, Swartz uses quantitative approaches in cell biology and physiology, including biotransport and biomechanics, to investigate the role of the lymphatic system in physiology and pathophysiology. She is particularly interested in the roles of the lymphatic drainage in regulating immunity as well as lymphangiogenesis in inflammation and adaptive immunity, particularly in cancer. Her lab approaches these fundamental questions with new tools and methodologies developed in the lab, including tissue engineered model systems, and also applies this knowledge to develop novel immunotherapeutic approaches in cancer, including lymph node-targeting vaccine approaches.

Shizhen Emily Wang

Associate Professor,
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BIOGRAPHY

S. Emily Wang, Ph.D., is currently Associate Professor of Cancer Biology at the City of Hope Beckman Research Institute in California, USA. She first began her research career in the late 1990s as a virologist and obtained her doctorate from University of Nebraska-Lincoln and Nankai University in 2002, followed by post-doctoral trainings at Johns Hopkins University and Vanderbilt University studying Cancer Biology. Dr. Wang's lab at City of Hope is among the first to carry out de novo sequencing of circulating small RNA in breast cancer patients and has discovered various functions of cancer-derived extracellular miRNAs in cancer–host crosstalk.

Richard Mark White

Assistant Member, Department of Cancer Biology & Genetics,
Memorial Sloan Kettering Cancer Center, New York, US

BIOGRAPHY

Dr. Richard White is an Assistant Member in the Department of Cancer Biology & Genetics at Memorial Sloan-Kettering Cancer Center and an Assistant Professor at Weill-Cornell Medical College. After training as an M.D./Ph.D. at Albany Medical College, he pursued clinical training in Internal Medicine at Yale-New Haven Hospital, where he also served as a Chief Resident. This was followed by a medical oncology fellowship at the Dana-Farber Cancer Institute/ Massachusetts General Hospital. He did his postdoctoral research work in the laboratory of Dr. Leonard Zon at Children's Hospital Boston and the Harvard Medical School, where he pioneered the use of zebrafish as a model to study cancer. In recent years, the zebrafish has emerged as an increasingly important model for cancer biology because of its unique capacity for screening of new cancer genes and pathways. At Sloan Kettering, he sustains a highly successful laboratory program while maintaining a clinical presence. His research focuses on the mechanisms by which cancers, especially melanoma and pancreatic, acquire metastatic capacity, the cause of nearly all cancer deaths. He uses the zebrafish for these studies because they are optically transparent, and breed in large numbers, allowing him to take unbiased approaches to find novel drivers of metastasis. Combined with its strengths in transgenic technology and in vivo imaging, he has addressed the genetic underpinnings of metastasis, considering both the tumor cell as well as the host genetic background. His goal is to identify the molecular mechanisms that allow tumors to evolve and to determine whether such mechanisms could be exploited therapeutically.

Max S. Wicha

Madeline and Sidney Forbes Professor of Oncology
Founding Director Emeritus,
University of Michigan Comprehensive Cancer Center,
Ann Arbor, US

BIOGRAPHY

Dr. Max Wicha is the founding director of the University of Michigan Comprehensive Cancer Center (UMCCC). Under his direction, the Cancer Center established itself as a national leader. It has received more funding from the National Cancer Institute than any other university-based center. Dr. Wicha is also a leader in breast cancer research and has been a pioneer in the field of cancer stem cells. According to the science citation index, he is among the most highly cited investigator in this field. Dr. Wicha's group was part of the team that first identified cancer stem cells in human breast cancers, the first in any solid tumor. His laboratory has developed many of the techniques and assays used to study these cells and to elucidate the pathways which regulate their behavior. These pathways have provided targets for the development of drugs aimed at targeting cancer stem cells. The UMCCC has established itself as a world leader in cancer stem cell biology and therapeutics and is currently conducting nine early phase clinical trials aimed at targeting cancer stem cells, the most of any center in the world. Dr. Wicha is co-founder of OncoMed Pharmaceuticals, a biotech technology company focused on developing cancer stem cell therapeutics, which has produced five agents currently in clinical testing.

Dr. Wicha is also a practicing clinician whose practice is focused on women with breast cancer. He also serves on advisory boards of a number NCI cancer centers and several biotechnology companies.

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The Role of Cancer Stem Cells in Breast Cancer Metastasis

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Breast cancer is a leading cause of mortality in women worldwide with a high incidence of recurrence and metastasis predominantly due to chemoresistance. The "cancer stem cell hypothesis" has raised new insights into stem cell biology implying that tumours originate from a population of cells termed cancer stem-like cells (CSLCs) through dysregulated self-renewal processes, which are highly tumourigenic and resistant to therapeutic regimes. Our current conventional therapies are based on eliminating differentiated tumours, whereas development of more effective therapeutics may actually entail targeting CSLCs. CSLCs as well as cancer cells demonstrate an intensive metabolic shift using higher rates of glucose utilisation leading to rapid tumour progression. Therefore, this project is aimed at reducing glycolysis using a variety of glycolytic inhibitors on the expression levels of two main stem cell markers OCT4 Pseudogene 1 (POU5F1B) and SOX2 in stem cell population of breast cancer cells. Expression of pluripotency markers was examined and it was found that SOX2 is more expressed in fructose-adapted cells and intriguingly OCT4A is not present in breast cancer cell lines. However, POU5F1B was expressed in some breast cell lines implying a possible role in carcinogenesis. Moreover, this study, by using a mammosphere formation assay, demonstrated that a fructose-adapted MCF-7 breast cancer cell line forms mammospheres significantly more than glucose-adapted cells indicating that, surprisingly, CSLCs do not need a high rate of glycolysis to survive/be oncogenic as long as they have sufficient sugar to grow. Modulation of this sugar adaptation could be a promising approach to stop or slow down CSLCs growth.

Unravelling Evolutionary Complexity and Selection in Prostate Cancer to Bone Metastasis

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Recent discoveries have highlighted the roles that tumour heterogeneity and evolution have in explaining successful metastases as well as treatment resistance. All this points to the importance of understanding how Darwinian evolution drives these processes. But while substantial advances have been made in our understanding of some of the key genetic drivers, much needs to be done to understand the selection pressures in metastasis. This lack can be explained in part by the absence of relevant models in which to explore cancer evolution. In prostate cancer to bone metastases, tumour cells interact with a variety of bone cells (messenchymal stem cells, osteoclasts, osteoblasts, macrophages, etc) through a variety of signalling molecules (TGF-Beta, RANKL,...), a complex system that we need to understand but that is difficult to capture with experimental models alone. We will present a computational model that integrates experimental and clinical data to explore how the interactions between phenotypically heterogeneous prostate cancer cells and the bone microenviroment capture key selective forces that explain tumour evolution and treatment failure. Using a first-principles/bottom-up computational model, parameterised using extant experimental literature as well as purposely designed experiments and clinical data we show how our model allows us to understand successful metastases and how they evolve resistance to conventional and novel treatments. Importantly we will show experimental validation for our results.

Gas6 Downregulation in the Bone Marrow by the Primary Tumor Induces Dormancy of Disseminated Breast Cancer Cells (DTCs)

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DTCs in the bone marrow (BM) can adquire a quiescent state for long periods of time to survive cancer therapies. Growth arrest-specific gene 6 (Gas6) can induce proliferation of breast cancer cells. We study if Gas6 levels in blood and BM of mice and breast cancer patients can exert an influence on the proliferative state of DTCs. GFP+4T1 murine breast cancer cells were orthotopically implanted into the mammary gland of syngeneic wt or Gas6-/- mice, being treated with 3mg/kg doxorubicin. Numbers and proliferative state of DTCs were determined by flow cytometry. Gas6 levels were measured by ELISA.

Decreased Gas6 levels were found in the BM plasma of tumor bearing compared healthy mice (3499±165vs.1593±188 pg/ml; n=18/15; $p<0.0001$). Similarly, we found lower Gas6 levels in the BMP of breast cancer patients compared to healthy BM donors (1210±349 vs. 5789±1566 pg/ml; n=5/47; $p<0.0003$). We concluded that primary tumors might elicit dormancy of DTCs by downregulating Gas6 in the BM. DTCs number per 5x105 total BM cells in Gas6-/- mice was less compared to wt mice (58±9 vs.127±16; n=10/10; $p<0.05$). Lack of Gas6 induced dormancy of DTCs because we found less GFP+BrDU+ cells in Gas6-/- mice in comparison to controls (18±1.94 vs. 35±4.73%; n=9/8; $p<0.03$). Dormancy was induced specifically in DTCs, because the proliferation state of normal BM cells wasn´t different between genotypes. This effect wasn´t due to differences in cell death because equal numbers of DTCs were in apoptotic or necrotic state in both genotypes. Enhanced dormancy of DTCs rendered them resistant towards chemotherapy because doxorubicine was killing less DTCs in Gas6-/- compared to wt mice (70±4.28 vs. 46±3.8%; n=9/9; $p<0.002$). Gas6 BMP levels were higher in breast cancer patients harbouring DTCs compared to those without DTCs, thus Gas6 seems to influence DTCs in humans as well. Our data indicate that Gas6 represents a regulator of DTC dormancy and chemoresistance in mice and humans.

The STAT3 Inhibitor Galiellalactone Effectively Reduces Tumor Growth and Metastatic Spread in an Orthotopic Xenograft Mouse Model of Prostate Cancer

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Background and Aims
Constitutively active STAT3 (pSTAT3) has been correlated to prostate cancer (PCa) progression and disruption of its signaling pathway represents a promising strategy for the treatment of patients with advanced PCa (Hellsten R, 2008). Galiellalactone (GL), a STAT3 inhibitor, reduces proliferation and induces apoptosis of DU145 cells, thus representing an interesting therapeutic agent against castration-resistant PCa (CRPC) expressing constitutively active STAT3 (Hellsten et al., 2008). Using in vitro techniques we have explored the effect of GL on PCa cell viability, apoptosis and invasion. Moreover, we have studied the effect of GL on tumor growth and metastatic spread in an *in vivo* orthotopic mouse model of PCa.

Methods
Cell viability was determined by MTT assay; apoptotic cells were visualized with M30 CytoDeath antibody 24h after treatment with GL. Cell invasion was analyzed using matrigel pre-coated cell culture inserts in the presence or absence of GL. For the animal study, NMRI nude mice were injected orthotopically with 1x106 DU145-Luc cells, and treated IP with GL every day. Primary tumor growth and metastatic spread were evaluated weekly by *in vivo* luminescence using the IVIS Lumina II system. Nine weeks after injection, animals were sacrificed and organs were analyzed for the presence of metastases. Primary tumors, regional lymph nodes (RLN) and distal lymph nodes (DLN) were also analyzed by immunohistochemistry for the presence of tumor-derived cells, apoptotic cells and actively proliferating cells.

Results
The treatment with the STAT3 inhibitor GL resulted in the inhibition of DU145 and DU145-Luc cell viability and invasion and in apoptosis induction *in vitro*. *In vivo*, GL reduced tumor growth and metastatic spread to RLN and DLN, while it induced apoptosis in the primary tumor.

Conclusions
Our results confirm that the inhibition of the STAT3 pathway is a promising therapeutic approach for the treatment of metastatic PCa. We found that GL is a good candidate for the treatment of this disease, as it reduces tumor growth and metastatic spread to lymph nodes.

Targeting of Melanoma Cells and their Associated Immune Environment with dsRNA-Based Nanocomplexes

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Cutaneous melanoma is the most lethal form of skin cancer, characterized by a remarkable ability to evade immune surveillance. Current immunotherapies against melanoma have focus on deactivation of intrinsic mechanisms of protection against immune recognition. Unfortunately, responses to these treatments are limited to a restricted subset of melanoma patients. Therefore, there is still the need to find alternative strategies to attack tumor cells and their microenvironment. Tumorassociated macrophages (TAMs) are an attractive target for their impact in melanoma development and metastasis. Here we present a new dsRNA nanocomplexes-base drug that can both promote tumor cell death, blunt lymphangiogenesis and shift macrophages polarization favoring tumor cell clearance, without affecting normal cells. These effects were accompanied by a potent Type I IFN response, more than 1000-fold stronger than with naked dsRNA. Moreover, combined treatment with anti-PDL1 blocking antibodies showed strong additive effects on tumor response, indicating dsRNA-based nanocomplexes as candidates for melanoma immunotherapy.

MIF-Expressing Pancreatic Cancer-Derived Exosomes Induce Kupffer Cell-Mediated Pre-Metastatic Niche Formation in the Liver

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Pancreatic cancers (PC) are highly metastatic with poor prognosis, mainly due to delayed detection. Here we show that pancreatic cancer-derived exosomes induce the formation of pre-metastatic niches in the liver, a process essential for pancreatic cancer metastasis to this organ. Pancreatic cancer-derived exosomes preferentially accumulate in the liver, and are specifically uptaken by von Kupffer cells. Once incorporated by these cells, exosomes induce the activation of pathways associated with liver fibrosis, upregulating transforming growth factor beta (TGF-ß). In response to elevated levels of TGF-ß, hepatic stellate cells increase fibronectin deposition, thus promoting extracellular matrix (ECM) remodeling. In turn, bone marrow-derived macrophages accumulate in the liver in a fibronectindependent manner, establishing the pre-metastatic niche. Interestingly, the analysis of livers isolated from spontaneous animal models of pancreatic cancer at various stages of disease progression revealed that pre-metastatic liver formation starts early during disease development, specifically at the pre-tumor stages (such as PanIN). A detailed proteomic analysis of pancreatic cancer exosomes revealed that macrophage migration inhibitory factor (MIF) is a key player mediating the effects of pancreatic cancerderived exosomes in inducing liver pre-metastatic niche formation. Strikingly, we found that MIF was markedly elevated in exosomes isolated from stage I pancreatic cancer patients that developed metastatic disease when compared to those in which the pancreatic disease did not progress, indicating that MIF may be a prognostic marker for the development of pancreatic cancer liver metastasis.

Clinical Impact and Molecular-Genetic State of Haematogenously and Lymphatically Disseminated Lung Cancer Cells

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Lung cancer is still the leading cause of cancer related mortality accounting for 1.4 million deaths per year worldwide with non-small-cell lung cancer (NSCLC) representing approximately 80% of all lung cancer deaths (1). Even patients with stage I disease have 5-year survival rates of only 60-70% (2). We have previously shown for breast, esophageal and prostate cancer that dissemination occurs early during cancer progression (3,4). Here, the harbingers of cancer spread, single disseminated cancer cells (DCCs) are associated with poor survival when detected in bone marrow (BM) or lymph nodes (LN).

Here we investigated systemic cancer spread in 119 early stage non-small-cell lung cancer patients undergoing surgery. We determined the extent and the impact of lymphatic and haematogenous tumour cell dissemination using antibodies against epithelial cell adhesion molecule (EpCAM) and cytokeratins 8/18/19, respectively. Individual cells were isolated from LN and BM and single cell comparative genomic hybridization (CGH) was used for molecular analysis of DCCs and primary lung cancer areas. The patients were followed for median 42 months (range 1-139) and Kaplan-Meier and Cox-regression analyses were performed to predictoverall survival. DCCs were detected in 24% of the LNs and 31% of the BMs. Survival analysis of the total patient group revealed that detection of DCCs in LNs was significantly associated with shorter survival, while that of BM-DCCs was not (logrank test $P < 0.001$ and $P = 0.332$, respectively). However, in the subgroup of patients with evident LN involvement (UICC stage N1) detection of BM-DCCs was the most important risk factor for reduced overall survival ($P < 0.001$).

Genetic analysis identified differences between DCCs from BM and LN for copy number alterations with LN-DCCs and primary tumors (median aberrations per sample 1.0, and 12.0 and 11.0, respectively; Kruskal-Wallis test, $P < 0.001$) displaying significantly more aberrations than BM-DCCs. Although BM-DCCs did not show any characteristic genomic patterns, we identified recurrent alteration in LN-DCCs. These changes were rarely detected in corresponding primary tumors indicating significant heterogeneity among systemic and local disease.

In summary, both LN-DCCs and BM-DCCs impact on the prognosis of lung cancer patients. Our molecular analysis reveals that cancer cells disseminated by the way of blood or lymph evolve independently and differently and differ from primary tumours. Therefore, the development of efficient adjuvant therapies will require a deeper molecular understanding of shared therapy target structures.

References
1- Jemal A, Bray F, Center et al.. CA Cancer J Clin. 2011;61:69-90.
2- Goldstraw P, Crowley J, Chansky K, et al. J Thorac Oncol. 2007;2:706-14
3- Husemann Y, Geigl JB, Schubert F, et al. Cancer Cell. 2008;13:58-68.
4- Schardt JA, Meyer M, Hartmann CH, et al. Cancer Cell. 2005;8:227-39

Biological Impact of Simple O-Glycans in Gastric Cancer Using Glycoengineered Cells

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Altered glycosylation is a common feature of cancer[1] and aberrant expression of sialylated structures, such as sialyl-Tn (STn), at the surface of cancer cells has been extensively reported and correlated with an invasive phenotype in various cancer models[2,3]. STn expression has been shown to be an independent indicator of poor prognosis in gastric cancer[4].

Glycoengineered gastric "SimpleCell" (SC) models lacking core-1 synthesis due to knockout of COSMC chaperone were used as a strategy to characterize the O-glycoproteome of two gastric cancer SC lines and to evaluate the biological impact of truncated O-glycans (Tn and STn) expression in gastric cancer cells.
COSMC KO cells allowed the identification of the O-glycoproteoma (499 O-glycoproteins and 1236 O-glycosites) of gastric cancer cells. Some of the identified glycoproteins constitute potential biomarkers of cancer and were also identified and validated in the sera from gastric cancer patients. These identified circulating O-glycoproteins modified with the STn glycoforms were confirmed to have an origin in the gastric tumour[5].

The glycoengineered cancer SimpleCells were also used to evaluate of the role of truncated O-glycans in the gastric cancer cell biology. Major cellular morphological alterations, signaling pathways activation, increased cellular migration and invasion were observed in STn overexpressing cells.

Overall, cancer cells expressing high levels of STn revealed a more aggressive phenotype, suggesting that expression of immature O-glycans and absence of further extension of O-glycans have a crucial impact in key cellular signaling pathways controlling cell behavior and therefore driving tumor cell aggressiveness.

1.Pinho,SS, et al. Trends Mol Med, 2013. 19(11):664-76
2.Marcos,NT, et al. Cancer Res, 2004. 64(19):7050-7
3.Pinho,S, et al. Cancer Lett, 2007. 249(2):157-70
4.Werther,JL, et al. Int J Cancer, 1996. 69(3):193-9
5.Campos D, et al. Mol Cell Proteomics, 2015. 14(6):1616-29

Immunohistochemical Markers of Distant Metastasis in Laryngeal and Hypopharyngeal Squamous Cell Carcinomas

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Introduction: Metastasis remains a major cause of mortality in head and neck squamous cell carcinoma (HNSCC). Current clinicopathological features have shown limited predictability for the risk of distant metastasis in individual patients, and therefore more accurate and reliable markers are needed. The aim of this study was to investigate the ability of various molecular markers present in primary tumors to predict the risk of developing distant metastasis.

Material and Method: Restrictive clinical criteria were applied for patient selection in order to carry out a case-control study with comparable clinical features on a group-wide basis and a similar risk of metastasis. All patients were surgically treated (with postoperative radiotherapy when appropriate) and classified as stage IV disease. Immunohistochemical analysis was performed for a panel of proteins known to participate in cellular processes relevant to metastatic dissemination (E-cadherin, annexin A2, cortactin, FAK, EGFR, p53, and p-AKT).

Results and Discussion: Results showed that the loss of E-cadherin expression was significantly correlated with the risk of distant metastasis ($P = 0.002$; log-rank test), while the loss of annexin A2 expression was nearly statistically significant ($P = 0.06$). None of the other protein markers assessed were associated with the development of distant metastasis.

Conclusion: According to our data the loss of epithelial adhesion seems to play a central role in the development of metastasis in HNSCC, and more importantly, immunohistochemical assessment of key proteins involved in cell adhesion regulation, such as E-cadherin could represent a useful tool to evaluate easily and routinely the metastatic potential of these carcinomas. This determination could be easily and routinely performed on standard paraffin-embedded pathology specimens, making feasible the inclusion of this molecular marker in the diagnostic work-up and treatment planning of these tumors.

Modulation of The Metastatic Lymph Node Niche by Melanoma Cells Through Secreted Exosomes

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Metastasis is the most devastating phase of tumor progression. Recent research has demonstrated that microvesicle-based information transfer by exosomes plays a key role in tumor progression. Melanoma-secreted exosomes have been demonstrated to home to the lymph nodes (LNs). Exosomes derived from highly metastatic models (B16-F10) have a faster and wider distribution in the lymphatic system as compared to exosomes derived from low (B16-F1) or non-metastatic model (melan-a) whose distribution is restricted to the sentinel lymph node. Our data demonstrate that exosomes secreted by highly metastatic models promote vascular leakiness in the sentinel lymph node in a short time range. Another main consequence of in vivo exposure of B16-F10-derived exosomes is the promotion of new lymphoangiogenesis in sentinel and distal LNs. In order to identify primary cell targets of melanoma-secreted exosomes we have performed confocal imaging and flow cytometry analysis. Our findings indicate that there exists a primary uptake of exosomes by lymphatic endothelial cells (LECs) from the lymph nodes. In vitro, human LECs also incorporate exosomes displaying a faster and increased uptake of SK-Mel103 than melanocyte-secreted exosomes. RNAseq and RT-PCR analysis of human LECs incubated with melanoma-derived exosomes confirms upregulation of lymphoangiogenesis-related genes together with the positive enrichment of molecular adhesion-, autoimmune- and cytokinerelated signatures among others. Our results support a role of tumorsecreted exosomes in promoting cellular and molecular alterations in the lymph node microenvironment fostering metastasis.In addition, analysis of exosome content in human lymphatic fluid suggest that proteomic cargo and particles are increased in melanoma patients opening the possibility of the use of circulating vesicles in lymphatic fluid as biomarkers and as a source of novel markers in pre-metastatic sentinel lymph nodes that predict relapse and metastatic potential.

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Genome Wide Loss-of-Function Screening to Identify Dormant Metastasis Genes in ER Positive Breast Cancer

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Breast cancer is the most frequently diagnosed cancer and remains the second leading cause of death among women in Europe and United States. Majority of cases of patient death are caused by metastatic relapse. In the ER positive breast cancer, tumors are characterized by the late appearance of metastasis, years or decades after primary tumor resection. In this setting metastatic dormancy plays a relevant role. The identification of mechanisms that allow dormant metastases to become active is essential for understanding the biology of ER positive breast cancer metastatic latency and has putative implications for clinical practice. Therefore, we aim to establish xenograft model of dormancy in ER positive breast cancer to bone metastasis and find molecular mechanisms responsible for metastatic dormancy.

Using in vivo selection in mice we isolated bone metastatic derivative population from poorly agressive parental cells. DBM cells survive in bone as dissaminated tumor cells and after extended periods of time form micrometastatic lesions which rarely progess into X-ray detectable osteolythic macrometastasis. This frequent and prolonged bone residency is opposite to the standard 3 weeks metastatic relapse that has been reported for breast to bone metastasis models. Moreover, it mimics metastasis latency reported in patients. Next, we performed genome-wide loss-of-function screening and in result we identified candidates for dormant metastasis genes in ER positive breast cancer.

Elucidating the Dormant Niche of Disseminated Tumor Cells in the Liver

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Background/Aims. Recently, we established that dormant disseminated tumor cells (DTCs) reside surrounding stable microvasculature in the lung and bone marrow. Although liver is also a common site of breast cancer metastasis, little is known about regulation of DTC dormancy in this organ. As such, we aim to determine whether dormant DTCs preferentially occupy a perivascular niche within the normal liver, and to define the cellular, architectural and biochemical properties of this niche.

Methods. Labeled mammary cancer cells (4T07) were injected orthotopically into female BALB/c (n=6) mice. Tumors were resected (V=250mm3) and mice sacrificed 6wks later. Immunofluorescence staining for GFP/ki67 in combination with CD31, GFAP, vimentin or IbaI was performed to define the cellular composition/location of the liver dormant niche. An organotypic model of the liver's microvascular niche (MVN) consisting solely of human liver cells was developed to assay the effect of liver microvasculature on breast tumor cell outgrowth.

Results. 4T07 cells disseminated to liver without causing overt metastases. Single, dormant 4T07s (GFP+/ki67-) were commonly detected within the sinusoids (38%), and extravasated (62%) in a perivascular niche. Dormant (77%) and proliferative (75%) 4T07s were in direct contact with quiescent GFAP+ hepatic stellate cells (HSCs). This profile altered with HSC activation. The majority of 4T07s were in direct contact with IbaI+ Kupffer cells. Breast tumor cell outgrowth was significantly inhibited in MVN cultures vs. liver stroma alone (p=0.0103). Dormant clusters were significantly increased in liver MVNs (vs. stroma, p=0.0253).

Conclusions. Our preliminary data indicate the dormant niche within the liver is also vascular, with an intrasinusoidal and extravasated locale. Further work will delineate whether disruption of the perivascular niche is sufficient to facilitate metastatic outgrowth, and the molecular mechanisms involved in DTC dormancy and reemergence.

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Increased Expression of Gasdermin B Predicts Poor Prognosis and Response to Therapy in HER2-Positive Breast Cancer

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Around 20% of breast carcinomas are characterized by the amplification and over-expression of the tyrosine kinase receptor ERBB2 oncogene. As HER2-positive tumors are commonly aggressive aggressive cancers traditionally associated to poor prognosis, especially when they metastasize to distant organs. The clinical outcome of these tumors has gradually improved due to the development of various therapeutic strategies directed against HER2. However, a high percentage of HER2-positive cases do not show substantial clinical benefit from current standard targeted therapy and the mechanisms underlying resistance remain partially unknown. Interestingly, among the potential mediators of resistance, ERBB2 is frequently co-amplified and co-expressed with neighbor genes on chromosome 17q12-21. We recently demonstrated that GSDMB over-expression promotes cell motility, invasion and metastasis in breast carcinoma cell lines, and GSDMB expression was found to be upregulated in breast cancer samples. However, the potential association with the HER2-positive subtype remains unknown. This study analyses the role of the gasdermin B (GSDMB) gene in this region, whose product has been shown to mediate breast cancer invasion and metastasis.

Patients and Methods

GSDMB expression in independent breast cancer microarray datasets was assessed for its correlation with pathological and clinical parameters. Tissue microarrays containing neoadyuvant and adyuvant HER2-positive breast tumors were analyzed for the association between GSDMB gene amplification and expression, HER2 status, disease relapse and residual cancer burden.

Results

In all analyses, GSDMB gene amplification and/or over-expression strongly correlated with HER2-positive breast cancer. GSDMB expression in these tumors was significantly associated with poor prognosis. Gene amplification and over-expression in tumors that underwent neoadjuvant or adjuvant therapy revealed significant associations with disease relapse, metastasis and poor response to trastuzumab therapy.

Conclusion

This study identifies the ERBB2 co-amplified and co-expressed gene GSDMB as a critical determinant of differential prognosis and predictive factor to trastuzumab-based therapy in HER2-positive breast cancer.

Adipocyte-Derived Exosomes Promote Melanoma Progression: a Novel Paradigm for The Link Obesity and Cancer

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We and others have recently demonstrated that mature adipocytes, the main cellular components of adipose tissue (AT) participate in tumor progression by secreting proteases and pro-inflammatory cytokines. Understanding the role of adipocytes in cancer is of major clinical importance as it is now largely acknowledged that obesity, where the normal balance of adipose tissue secretions is perturbed, affects cancer incidence and prognosis. Research into the signals underlying the communication between adipocytes and cancer cells has been limited to analysis of the role of secreted soluble factors. Recent evidence demonstrates that exosomes play a pivotal role in local and systemic cell-cell communication in cancer. Very little data are available concerning the composition and function of exosomes-secreted by adipocytes, and these elements have never been investigated in a cancer context. We explored for the first time the effect of these vesicles on melanoma progression, a tumor type adversely affected by obesity. We show that mature adipocytes secrete large amounts of exosomes, which are uptaken by tumor cells leading to increased migration and invasion (but not proliferation). Using mass spectrometry, we analyzed the adipocyte exoproteome. Interestingly, these vesicles carry many proteins implicated in fatty acid -oxidation (FAO), a feature that is highly specific to adipocytederived exosomes. Inhibition of this metabolic pathway in tumor cells completely abrogates the increase in migration/invasion observed in the presence of adipocyte-derived exosomes. Very interestingly, adipocytes isolated from obese individuals (that present both quantitative and qualitative modifications) possess a higher ability to stimulate tumor aggressiveness that is also dependent on a metabolic remodeling towards FAO in tumor cells. This study emphasizes for the first time the role of adipocytes-derived exosomes in cancer progression and its regulation by obesity.

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Regulation of Lung Adenocarcinoma Differentiation by RIP4

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Cancer metastasis is a multistep process facilitated by alterations of tumor differentiation through loss of cell polarity and extracellular matrix remodeling. Our work identified Receptor-Interacting serine/threonine- Protein Kinase 4 (RIP4) as a novel regulator of tumor differentiation in lung cancer. Bioinformatics analyses of human lung adenocarcinoma samples showed that poorly differentiated tumors express low levels of RIP4, whereas high levels are associated with better overall survival. Tumorspecific Rip4 knockdown in an autochthonous mouse model of lung adenocarcinoma, initiated by KrasG12D expression and loss of p53, progressed to a poorly differentiated state, with increased levels of markers such as multiple matrix-metalloproteinases, lysyl oxidase and Hmga2 as determined by gene expression profiling. In vitro analyses showed that RIP4 knockdown confers an increased anchorage independent growth, and that RIP4 prevents the interaction between the pro-oncogenic transcription factors NF-κB and STAT3, a finding confirmed in tumors in vivo. Altogether, our results indicate that RIP4 prevents the progression of lung adenocarcinoma to a more aggressive and poorly differentiated state, possibly through inhibiting important interactions between NF-κB and STAT3.

Aspirin Affects Early Steps of Metastasis Through the Inhibition of COX-1

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Background
Metastasis is the major cause of cancer related mortality, due to a poor understanding of the metastatic process and a subsequent lack of specific anti-metastatic therapies. Evidence from experimental studies and clinical trials shows that aspirin reduces the incidence of distant metastases and cancer mortality. It is well established that aspirin inhibits cyclooxygenase (COX)-1 and COX-2, triggering anti-thrombotic and anti-inflammatory effects, respectively. However, the mechanisms underlying the anti-metastatic effect of aspirin are still largely unknown.

Methods
We have employed an experimental model of metastasis in which tumour cells are intravenously injected in syngeneic mice treated with aspirin or with a selective COX-1 inhibitor (SC-560). Metastatic lung nodules, tumour cell survival, protein expression in lung sections and the interaction between tumour cells, platelets and monocytes/macrophages are then evaluated.

Results
We show that aspirin and SC-560 dramatically reduce the number of metastatic lung nodules in our model. Serum levels of TXB2 (a biomarker of COX-1 activity) confirmed COX-1 inhibition by either treatment, indicating a pivotal role of this isoform in the metastatic process. COX-1 inhibition by aspirin or by SC-560 was found to impair the survival of tumour cells within the vasculature and treatment carried out during the first hours of their dissemination was sufficient to reduce metastasis. This effect is partly due to a reduced platelet association on tumour cells, which leads to a decreased endothelial activation and recruitment of metastasis promoting monocytes/macrophages to tumour cells.

Conclusions and future perspectives
Our data suggest that the mild anti-coagulant effect of aspirin derived from COX-1 inhibition is sufficient to exert an anti-metastatic effect. In future work we aim to further characterise the role of COX-1 in the development of metastasis.

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NOTCH1-Reliant Tumor Vessel Normalization by Chloroquine

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The old antimalarial drug Chloroquine (CQ) is exploited in clinical trials to potentiate anti-cancer therapy, based on its ability to block the pro-survival autophagy in cancer cells. However it is unknown if CQ solely acts by inhibiting autophagy and whether it also affects the tumor microenvironment. In this study we found that besides blocking cancer cell growth, CQ also affects the endothelial cells (ECs), hereby normalizing the tumor vasculature. This vessel normalizing effect of CQ reduced tumor hypoxia, cancer cell intravasation and metastasis, while improving the delivery and response to chemotherapy. By compromising autophagy in melanoma cells or using mice with a conditional knockout of the essential autophagy gene ATG5 in endothelial cells, we found that the favorable effects of CQ on the tumor vasculature did not rely on autophagy. Instead CQ-induced vessel normalization relied mainly on altered endolysosomal trafficking and sustained NOTCH1 signaling in endothelial cells. Indeed these CQ-mediated effects on the tumor vasculature were abrogated when tumors were grown in mice harboring endothelial cell-specific deletion of NOTCH1.

This study identifies CQ as the first clinically-available vessel normalizing chemical compound. This is important for the cancer field, where vessel normalization rather than pruning is emerging as the preferred therapeutic approach. Tumor vessel normalization ameliorates tumor perfusion, hereby rendering the cancer cell less aggressive and ameliorating chemodelivery within the tumor, resulting in more efficient chemotherapy. In addition, tumor vessel normalization also increases the barrier function of the vessels, which reduces the access of cancer cells to the circulation, hereby preventing metastasis.

Heterogeneity of Cancer Stem Cell Markers Expression in Breast Cancer Primary Tumors and Lymph Node Metastases – Clinical and Biological Significance

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Cancer stem cells (CSC) are known to drive tumor growth, however detection of CSC markers expressing cells in clinical material might be hampered by tumor heterogeneity. It is also not well understood if heterogeneity of CSC markers in primary tumors (PT) is reestablished in metastases and what is the significance of such heterogeneity. The current work addressed these issues by analyzing levels of CSC markers in different areas PT of the breast and their lymph node metastases (LNM). PT (N=108) and LNM (N=52) were collected from stage I-III breast cancer patients. Protein levels of CSC markers (CD133, ALDH1, CD44, OCT4, NANOG) were analyzed by immunohistochemical staining in up to 5 spatially separated areas of PT and LNM. Level of heterogeneity of each marker expression was calculated with Gini Index (GI), grading samples from non-heterogeneous (GI sore 0) to highly-heterogeneous (GI score 1). Results of IHC analysis were correlated with clinico-pathological parameters, EMT markers expression and dissemination of circulating tumor cells. NANOG, ALDH1 and CD44 were the most abundant CSC markers in PT and LNM. Hierarchical clustering of protein expression revealed presence of 4 clusters in PT – mesenchymal NANOG+ (CD44+/NANOG+), mesenchymal NANOG- (CD44+/NANOG-), epithelial (ALDH1+/NANOG+) and neutral (NANOG+). A trend was observed towards more efficient CTCs dissemination from the mesenchymal NANOG- and neutral clusters (P=0.10). The least expressed markers in PT - CD133 and OCT4 showed one of the highest GI score in PT (0.413 and 0.403), but converted to low GI score in LNM (0.101 and 0.219), respectively; (P=0.0067 for CD133, P=0.09 for OCT4). At the same time average percentage of CD133 and OCT4 positive cells increased in LNM (43.4% and 36.3%) in comparison to PT (19% and 21.2%), respectively. CD133 positive status and its GI heterogeneity index in PT were linked with lack of hormone receptors as well as expression of the EMT marker vimentin (P<0.00001 for all).

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Gastric Cancer Circulating Exosomes: Detection and Relevance for the Disease Progression

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Gastric cancer is one of the leading causes of cancer-related mortality. New prognostic and predictive biomarkers are urgently needed. Exosomes are small vesicles secreted by most of cell types, related with cancer progression1. Exosomes increase the metastatic behavior of primary tumors by permanently 'educating' bone marrow progenitors through the receptor tyrosine kinase MET2. In this study we have evaluated the role of circulating exosomes in a prospectively recruited cohort of advanced gastric cancer (AGC) patients, including a subgroup treated with the new anti-MET antibody, Onartuzumab. MET and its ligand had been extensively related with GC progression3. We have evaluated the use of circulating exosomes to predict the efficiency of standard chemotherapy versus the combination with therapies anti-MET.

1. Exosomes: composition, biogenesis and function. . Clotilde Théry, Laurence Zitvogel & Sebastian Amigorena. Nat. Rev. Immunology. 2002. (2): 569-579.
2. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Peinado H et al . Nat Med. 2012. (6): 883-91.
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Src Signaling in Triple Negative Breast Cancer Cells: Role of Cyr61

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The SFKs (Src Family Kinases) control cellular pathways involved in division, motility, adhesion, angiogenesis, and survival. Therefore, their deregulation is associated with tumorigenesis, and metastasis. c-Src is overexpressed and/or aberrantly activated in epithelial tumors: pancreatic, colorectal, prostatic, ovarian, breast, etc. We previously showed that SFKs catalytic inhibitors (Dasatinib, PP2, and SU6656) reduce proliferation, migration, and invasiveness of MDA-MB-231. Here, we analyzed c-Src contribution to initial steps of metastasis by Tet-On conditional expression of a specific shRNA-c-Src, which suppressed c-Src mRNA and protein levels in MDA-MB-231. c-Src suppression did not alter cell proliferation or survival, but it significantly reduced anchorage-independent growth. Concomitantly with diminished tyrosine-phosphorylation/activation of Fak, caveolin-1, paxillin and p130CAS, c-Src depletion inhibited migration, invasion, transendothelial migration, and reduced MMP2, MMP7 and MMP9 in secretome. Quantitative proteomic analyses of secretome showed that Cyr61 levels, detected in exosomal fraction, were diminished upon shRNA-c-Src expression. However, Cyr61 expression was unaltered inside cells. Cyr61 partially colocalized with cis-Golgi gp74 marker, and with exosomal marker CD63, but c-Src depletion did not alter their distribution. In SUM159PT, transient c-Src suppression also reduced secreted exosomal Cyr61. Furthermore, conditional expression of c-Src dominant negative mutant (c-Src-K295M/Y527F) in MDA-MB-231 and in SUM159PT diminished secreted Cyr61 as well. Cyr61 transient suppression in MDA-MB-231 inhibited invasion and transendothelial migration. Finally, in both MDA-MB-231 and SUM159PT, a neutralizing Cyr61 antibody restrained migration. Collectively, these results suggest that c-Src regulates secreted proteins, including exosomal Cyr61, which are involved in modulating the metastatic potential of triple negative breast cancer cells.

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Protein Tyrosine Phosphatase PTPL1/PTPN13: A New Breast Tumour Suppressor Candidate

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Breast cancer is a major problem of the public health which the incidence continues to increase. Mortality is linked to the metastasis. PTPL1 studies, the largest Protein tyrosine phosphatase, have shown that it presents the characteristics of tumor suppressor gene and that it has a role in the signaling pathways involved in the motility and invasion. Our team has succeeded to establish an isogenic cellular model capable of expressing PTPL1 in inducible fashion in a highly invasive cell lines. Recently, we have shown that functional PTPL1 expression has a negative impact on migration and invasion and promotes cell-cell adhesion. Interestingly, the phosphatase dead mutant showed the same phenotype as the transfection control, this makes evidence that PTPL1 activity is crucial for the aggressiveness inhibition. Later on, we will test PTPL1 overexpressing clones tumorigenicity in vivo in mice.

Furthermore, we proposed to study the signaling pathways regulated by PTPL1. For this we opted for proteomic comparative approach, the SILAC. Our findings concerning PTPL1 signaling pathways show that PTPL1 regulates the phosphorylation of some cell-cell junction proteins as well as proteins involved in the regulation of the cell metabolism. Our ongoing work consists on immunoprecipitating these protein candidates in order to verify their phosphorylation status in the presence of PTPL1. In parallel, we are working on the immunoprecipitation of the substrate trapping mutant to seek for new substrates or partners for PTPL1 by proteomic approaches, the first trials gave an encouraging results. Finding out new substrates and decrypting new signaling pathways of PTP1 would contribute to the innovation of targeted therapy.

Over-Expression of the Oncostatin M Receptor in Squamous Cell Carcinoma Associates with Induction of EMT and Metastasis

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An important feature of cervical squamous carcinogenesis is genomic instability caused by deregulated expression of human papillomavirus oncogenes in proliferating epithelial cells. A genomic imbalance that is commonly selected in advanced cervical squamous cell carcinoma (SCC) is copy number gain of chromosome 5p. One gene that is likely to drive selection of this abnormality is the oncostatin-M receptor (OSMR; located at 5p13.1), which is commonly over-expressed in cervical SCC and associated with a significantly worse clinical outcome. Cervical SCC cells that over-express OSMR show enhanced responsiveness to the major ligand OSM, which induces multiple pro-malignant effects, including a pro-angiogenic phenotype and increased cell migration and invasiveness (1). Furthermore, activation of OSMR pathway in cancer cells leads to increased lung colonization *in vivo*. OSMR over-expression is associated with activation of epithelial-to-mesenchymal transition (EMT) and STAT3 and MAPK pathways and with induction of the pro-angiogenic factor VEGF. A further gene induced by OSM is transglutaminase 2 (TGM2), which interacts physically with integrin-α5β1 and acts as a co-receptor for fibronectin, so inducing cell migration and invasion (2). Together, these data indicate that OSM-OSMR pathway is of biological significance in SCC and a candidate for therapeutic targeting (1). Our most recent data suggest that OSMR pathway may also be important in the progression of triple negative breast cancer, where it predicts resistance to chemotherapy.

1. Caffarel MM, Coleman N. (2014) Oncostatin M receptor is a novel therapeutic target in cervical squamous cell carcinoma. Journal of Pathology 232:386-90
2. Caffarel MM, et al (2013) Tissue transglutaminase mediates the pro-malignant effects of Oncostatin M receptor over-expression in cervical squamous cell carcinoma. Journal of Pathology 231:168-79

Converging Effectors of Airway Specification, Regeneration, and Metastasis Initiation in Lung Cancer

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Lung cancers are biological heterogeneous malignancies with poor prognosis that metastasize to the brain. The underlying mechanisms of this heterogeneity and their biological consequences are poorly understood. Through an integrated approach, we examined the molecular relationship between epithelial differentiation states, airway injury, lung cancer subtypes, and clinical outcome (1). We identified a subset of metastatic lung adenocarcinoma (LUAD) defined by its expression of an alveolar lineage restricted transcriptional program with enrichment of specific extracellular matrix interacting proteins and proteoglycans. Mechanistically, this lineage program links the molecular effectors of pulmonary regeneration to the tumor re-initiating capacity of LUAD cells within specific niches in the lungs and brain.

Our findings suggest that epithelial lineage states intrinsic to the airways are determinants of metastatic competence in specific lung cancers. The implications of these findings for the origin(s) and treatment of LUAD metastasis will be further discussed.

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(1) Cheung, W.K.C., Zhao, M., Liu, Z., Stevens, L.E., Cao, P.D., Fang, J.E., Westbrook, T.F., and Nguyen, D.X. (2013). Cancer Cell, 23 (6): 725-738.

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Acquisition of Chemotherapy Resistance in Acute Myeloid Leukemia: Interplay Between Lipid Metabolism and VEGF Receptor Signalling

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The metabolic reprogramming plays a pivotal role in cancer progression. However, it is unclear if selective pressures imposed on cancer cells impact on the array of metabolic-adapted features that contribute for tumourigenesis.
We have established a new xenograft model of human AML (hAML), based in the intra-bone marrow injection of human erythroleukemia (HEL) cells on sublethally irradiated immunodeficient Rag2-/-gchain-/- mice, which suitably reproduce hAML growth, spreading and associated mortality. In this model, we applied a chemotherapy protocol to induce disease remissions, followed by predictable recurrences of hAML. HEL tumour cells from the bone marrow of mice subjected (chemo-selected bone marrow HEL, n=5) or not (untreated bone marrow HEL, n=7) to chemotherapy were isolated and the corresponding tumour cell line variants were established *in vitro*.
The metabolic profile of the chemo-selected and untreated ex-vivo HEL cell line variants was determined by 1H nuclear magnetic resonance and revealed increased levels of choline, pyruvate and myo-inositol in the chemo-selected HEL cell lines. Moreover, chemo-selected HEL cell lines also present higher levels of specific fatty acid residues (free and/or from phospholipids residues ω-CH3 and (CH2) n) and total intracellular lipids. Phenotipically, chemo-selected HEL cell lines present significantly higher levels of chemokine/growth factor receptors CXCR4, VEGFR-1,-2 and -3. We have previously shown that VEGF receptor signalling is associated with increase aggressiveness in AML [1] and that cholesterol regulates VEGF:VEGFR1 signalling in subsets of acute leukemia [2] Importantly, we now show that blocking VEGFR2 signalling in vitro sensitizes chemoselected HEL cell line variants to apoptosis by chemotherapy as measured by AnnexinV+; 7AAD+ staining by flow citometry. Our data suggests that the interplay between lipid metabolism and VEGFR signalling underlies the acquisition of chemotherapy resistance in AML.

[1] Fragoso, R., Pereira, T., Wu, T., Zhu, Z. Cabeçadas, J and Dias S. VEGFR-1 (FLT-1) activation modulates acute lymphoblastic leukemia localization and survival within the bone marrow, determining the onset of extramedullary disease. Blood, 2006. 107(4): p. 1608-16.
[2] Casalou, C., Costa, A., Carvalho, T., Gomes, A.L., Zhu, Z., Wu, Y. and Dias S. Cholesterol regulates VEGFR-1 (FLT-1) expression and signaling in acute leukemia cells. Mol Cancer Res, 2011. 9(2): p. 215-24.

Breast Cancer Cells Invasiveness is Modulated by GRK2

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Metastatic processes are dependent on the ability of cancer cells to penetrate the basement membrane and to remodel the Extracellular Matrix (ECM). We have identified G-protein-coupled receptor kinase 2 (GRK2) as a relevant driver of migration and invasiveness of breast cancer cells. GRK2 is a key node in signal transduction pathways; displaying a very complex interactome in both a kinase-dependent and independent manners. Alterations in GRK2 protein levels have been associated to several pathological conditions, such as inflammatory diseases or some type of tumors. We find GRK2 up-regulated in most of primary tumors of Infiltrating Ductal Carcioma (IDC) patients that underwent regional lymph node colonization. Moreover, lymph node metastasis derived from these tumors either retained or acquired enhanced GRK2 levels, arguing for an important role of GRK2 in the invasive process. Consistently, over-expression of GRK2 in different breast cancer cells favors their migration towards different stimuli such as Heregulin, fibronectin, S1P, IGF1 or CCL21, whereas its down-modulation strongly prevents this process. Moreover, GRK2 is required for invadopodia-dependent two-dimensional matrix proteolysis and for the breakdown of both basement membrane and ECM. Accordingly, silencing of GRK2 in the highly invasive MDA-MB-231 breast adenocarcinoma cells hampers cell invasion to matrigel and to collagen type I in 2D and 3D models of invasion. Overall, these results point at GRK2 as a key pro-invasive and pro-metastatic factor in tumoural cells and thus as a possible therapeutic target in breast cancer.

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Role of Soluble and Exosomal B7-H3

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The B7 family of immunoreceptors is considered essential in the regulation of the adaptive immune system, but many of their members are also expressed in non-hemopoietic cells and are emerging as important players in cancer. B7 proteins are divided into 3 different groups, according to the signals they transduce upon T cell activation: I) costimulatory; II) inhibitory (e.g B7-H1/PD-L1); and III) costimulatory/inhibitory (e.g. B7-H3). The B7 family members are type 1 transmembrane proteins with a cytoplasmic tail, a transmembrane region and an extracellular region. They are mostly localized to the plasma membrane where they interact with receptor partners in T cells, which mediate their immune modulatory functions, but their partners in non-hemopoietic cells are mostly unknown.

The human B7-H3 molecule is a heavily glycosylated protein which consists of 534 amino acids in its predominant longer form, but it is also present in a shorter isoform, as well as soluble isoforms. B7-H3 is overexpressed in malignant cells in many types of tumors. In contrast, the protein is not expressed, or is expressed at low levels, in most normal cells or tissues. B7-H3 protein expression has been linked to poor prognosis and resistance to therapy in many types of cancer, but the molecular basis for the functional roles of B7-H3 in cancer is poorly known. In this context, we have identified an oncogenic non-immunological role of B7-H3 in melanoma and breast cancer cells, which promotes metastasis and resistance to chemotherapy. These B7-H3 tumorigenic effects could be mediated by regulation of proteins in the Jak2/Stat3 signaling pathway, as well as by modulating the expression of cytokines and other metastasis associated genes. We detect B7-H3 in the membranes and nucleus of tumor cells, on circulating tumor cells, as well as on exosomes, and will present preliminary data on the role of soluble and exosomal B7-H3 in melanoma cell lines.

Non-Invasive Whole-Body Analysis of Premetastatic Niche Formation Identifies MIDKINE as a Novel Driver of Lymphangiogenesis and Metastasis in Melanoma

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Cutaneous melanoma is the deadliest form of skin cancer. Although the presence of tumor cells in lymph nodes is a poor prognosis indicator, the specific contribution of lymphangiogenesis to the mortality associated with melanoma metastasis remains unclear. This is mainly due to the lack of tumor markers and experimental models for non-invasive imaging of lymphangiogenesis in vivo. To address molecular mechanism(s) regulating metastatic spread of melanoma, we developed a series of genetically engineered mouse strains derived from a "lymphoreporter model" in which a luciferase cassette is expressed as a reflection of the endogenous Flt4 (VEGFR3) promoter. These "lymphoreporter" melanoma models allowed tracing of metastatic dissemination from very early stages of melanoma development. In particular, we have identified distinct patterns of melanomadriven neo-lymphangiogenesis activated at distal sites before the actual colonization by tumor cells. Proteomic analysis of the melanoma secretome revealed MIDKINE as a novel inducer of these pre-metastatic niches, with unexpected roles in the lymphatic vasculature. Importantly, long-term follow up analyses of disease free and overall survival in human melanoma specimens validated MIDKINE as a putative risk factor for melanoma metastasis. Together, these results support the VEGFR3-luc mouse melanoma models as a cost-effective strategy for gene discovery in the context of otherwise aggressive and intrinsically metastatic tumors.

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Identification and Therapeutic Targeting of Metastasis-Initiating-Cells in Human Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (SCC) is the 4th-5th most prevalent tumor in industrialized countries. It has a dismal prognosis, and severely affects the quality of life of patients. Resection of the tumor often requires aggressive surgery that removes large portions of the oral cavity. Approximately 50% of the patients develop lymph-node metastasis alone or in combination with lung metastasis. The prognosis of these patients is dismal with a 5-year survival below 20%. It is estimated that oral SCC will be the third most prevalent cancer within the next 30 years. We have developed an orthotopic model of patient-derived oral SCC that faithfully recapitulates the process of tumor growth and progression, including the development of lymph node and lung metastases. This model is allowing us to monitor and study the process of metastatic spread in an unprecedented detailed manner. We have identified a population of cells within the primary tumor that constitutes the cell of origin of metastasis (i.e. metastasis-initiating-cells). These cells are defined by a signature that allows them to survive and thrive in the lymph nodes and bronchoalveolar environments. Interestingly, this signature indicates that metastasis-initiating-cells use a distinct type of metabolism that is adapted to take advantage of the specific nutrient environment present in the lymph nodes. Importantly, inhibition of this pathway either by shRNA or through pharmacological means prevents metastatic spread of patient-derived SCC tumors. Activation of this pathway in non-metastatic tumors converts them into highly metastatic lesions. Analysis of public data indicates that the presence of this signature strongly correlates with poor prognosis in patients with oral SCC, but also in those with lung SCC, bladder cancer, ovarian cancer, or glioblastoma.

Exosomes Enriched in Stemness/Metastatic-Related mRNAs Promote Oncogenic Potential in Breast Cancer. Specific Prognostic Exosome Signature

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Purpose: Tumor-derived exosomes are a significant mechanism of intercellular communication, affecting tumor-related pathways, such as angiogenesis, drug resistance and immune response. This study analyzed the release of exosomes from breast tumor cells with different capacities of stemness/metastasis based on CXCR4 expression and evaluated their involvement in the capacity of generate oncogenic features in recipient cells.

Experimental design: Oncogenic effects of breast cancer cells-derived exosomes were evaluated in vitro and in vivo models. Exosome cargo in mRNAs involved in stem cell differentiation and metastasis were analyzed by specific quantitative PCR arrays. Finally, stemness- and metastaticrelated mRNA most differentially detected in exosomes were analyzed in plasma of breast cancer patients assessing its capacity as a prognosis marker.

Results: *In vitro*, exosomes released from CXCR4-tumor cells modify stemness markers, and proliferation, migration and invasion capacities of neighboring cells. Accordingly, inoculation of CXCR4-cells-derived exosomes in immunocompromised mice mediated primary tumor growth and metastatic potential. Exosomes isolated from CXCR4-cells and stem-like cells were highly enriched in stemness- and metastatic-related mRNAs and showed high homology between them. Furthermore, comparative analysis of mRNAs contained in exosomes isolated from patients revealed a gene signature of mRNAs highly enriched in exosomes of patients with worse prognosis.

Conclusions: Our data support the view that exosomes released by CXCR4-cancer cells that exhibit stem-like properties are capable to transfer these oncogenic features to recipient cells. In addition, our results show the involvement of "stemness and metastatic signature" mRNA in exosomes from plasma in breast cancer patient survival.

Circulating miRNA as Candidate Markers for Metastatic Medullary Thyroid Carcinoma Patients

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Medullary thyroid carcinoma (MTC) is a rare neuroendocrine malignancy originating from parafollicular C cell and accounts for 1-2% of all thyroid cancers. Distant metastases, that typically occur in lungs, bones and liver, are often multiple and are the main cause of MTC-related death.

In this study we have investigated circulating miRNA profiles associated with metastatic MTC by microarray analysis. We compared global miRNA expression in plasma samples from 19 metastatic MTC patients and from 26 matched healthy donors and we identified a signature of 21 plasmatic miRNAs significantly upregulated in MTC patients that are under validation by qRT-PCR. We obtained archival FFPE of primary tumors of the same patients and we performed miRNA profiling of tumoral tissues compared with normal thyroid defining a list of 44 significantly deregulated miRNAs. We identified one miRNA, among others over-represented in MTC patients' plasma, also up-regulated in primary tumors, thus suggesting that it is likely a MTC-derived specific miRNA. To evaluate if plasmatic miRNAs could be candidate biomarkers for monitoring therapy we obtained pre- and post- treatment paired plasma samples from a subset of patients (11 out of 19) treated with vandetanib. MiRNA microarray analysis didn't reveal any statistically significant deregulated miRNA induced after 2 months of therapy. We have thus defined the expression profiles of cellular and exosomal miRNAs induced by vandetanib treatment in the MTC cell line MZCRC1. After treatment, MTC cells secrete exosomes with miRNA cargo partially recalling cellular profile and also including specific additional miRNA. Interestingly, some miRNAs upregulated in MTC patients' plasma are also present in exosomes secreted by MZCRC1 cells.

This is the first report of circulating miRNA in MTC patients and our findings, although requiring further validation in a larger cohort, suggest plasmatic miRNAs as potential biomarkers for MTC.

Modulation of the Metastatic Niche in Peritoneal Carcinomatosis of Ovarian Cancer Via Mesothelial-to-Mesenchymal Transition

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Peritoneal dissemination is the primary metastatic route for ovarian cancer (OvCa), often accompanied by accumulation of ascitic fluid. Mesothelial cells (MCs) lining the peritoneal cavity can derive into carcinoma-associated fibroblasts (CAFs) through a mesothelial-to-mesenchymal transition (MMT) induced by cancer cells. Immunofluorescence staining of MCs from ascitic fluid of OvCa patients showed a MMT had taken place: positive expression of alpha-SMA (myofibroblast marker) and pSmad3 (downstream effector of TGF-beta1: key inducer of MMT). RNA-sequencing of ascitic fluid-derived MCs revealed upregulated genes related to EMT, CAFs, cytokine signalling and angiogenesis; and downregulated genes that most notably coded for tight junction-related proteins. Peritoneal implant biopsies of OvCa patients show alpha-SMA-positive CAFs expressing nuclear active pSmad3, but also calretinin, confirming their mesothelial origin. Surprisingly, pSmad3 was preferentially localized in the cytoplasm of cancer cells. The differential localization of pSmad3 was confirmed in vitro upon TGF-beta1 stimulation, suggesting that, despite producing large amounts of TGF-beta1, this pathway is truncated in OvCa cells, requiring MCs for environment transformation. Consequently, we pre-treated the peritoneum of mice and observed that tumour progression was significantly accelerated in MMT-induced mice; and blocking peritoneal TGF-beta1 receptor I maintained it at levels comparable to those of controls. Interestingly, subcutaneous co-injection of OvCa cells with ascitic fluid-derived MCs or with TGF-beta1-treated MCs showed significantly greater tumour growth compared to controls. Our work shows that CAFs derived from MCs through a MMT transform the peritoneum, creating a suitable metastatic niche for OvCa progression. We suggest the peritoneal environment could constitute an alternative target in the treatment of OvCa metastases, by strategically modulating the MMT with pharmacological agents.

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Melanoma Brain Metastasis is Facilitated by Instigation of Reactive Astrogliosis and Neuroinflammation

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Melanoma frequently metastasizes to the brain, resulting in dismal survival. A major obstacle for characterizing mechanisms of brain metastasis is the lack of tractable pre-clinical models that mimic the multi-stage process of brain metastasis. We established a novel model of spontaneous melanoma brain metastasis in immunocompetent mice. Aggressive local tumors are formed following orthotopic injection, and 3-4 months after surgical excision of the primary tumor, 50% of the mice develop brain macrometastases. By utilizing a unique ex-vivo modeling system, we detected brain micrometastases in 50 60% of the mice and quantified the metastatic load. Moreover, we show that the detection of melanoma transcripts in cerebrospinal fluid (CSF) is correlated with brain metastases. Interestingly, preliminary findings suggest that the melanoma-derived transcripts are found in exosomes. Astrocytes are glial cells that maintain brain homeostasis, and constitute a central part of the brain microenvironment. Reactive astrogliosis is the primary response of astrocytes to brain insult. While much data have accumulated on the contribution of astrogliosis to neurodegenerative diseases, the functional role of astrogliosis in promoting melanoma brain metastases is unknown. Utilizing our model we demonstrate early changes in the microenvironment and show that micrometastases were associated with activation of astrocytes and with hyperpermeability of the blood-brain-barrier. In-vitro, paracrine signaling by melanoma cells activated astrocytes to up-regulate a ‘wound healing program’ including Cxcl10 and Serpins. Finally, we show that intracranial co-injection of astrocytes with melanoma cells resulted in a 9 fold increase of tumor volume. Studying spontaneous melanoma brain metastasis in a clinically relevant setting that enables in-depth understating of the metastatic microenviromet is the key to developing therapeutic approaches that may prevent brain metastatic relapse.

Regulation of the Functional Interface Between Nucleotide Excision Repair and Transcription by MITF Modulates Melanoma Growth

Marcos Seoane[1,2], Julia Strauss[1,2], Ann-Christin Puller[1,2], Mashaallah Noshiravani[1,2], Susanne Feldhaus[3], Malik Alawi[4,7], Michael G. Kaul[5], Johanna M. Brandner[6], Jinyan Du[8], Jürgen Thomale[9], Peter J. Wild[10], Martin Zimmermann[11], Thomas Sternsdorf[1,2], Peter Nollau[1,2], Udo Schumacher[3], David E. Fisher[12], and Martin A. Horstmann[1,2],

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Survival, proliferation and differentiation of the melanocytic lineage are determined by the MITF. Mechanistically, it is still incompletely understood how MITF is linked to melanomagenesis. However genomic amplification of MITF and SUMOylation-defective MITF germline mutation has been identified in human melanomas. We observed that MITF controls the interface of nucleotide excision repair (NER) and transcription, through transactivation of GTF2H1, a TFIIH core element. Our results show that the NER/TFIIH complex is controlled by MITF in the presence and absence of UVR mediated genotoxic attack resulting in nucleotide repair deficiency and breakdown of global transcription upon MITF depletion. The association between MITF and GTF2H1 transcripts was confirmed utilizing GEOtranscriptome data on a large panel of primary and metastatic melanomas. Moreover, TMA analyses of 140 primary cutaneous melanomas shows that expression of GTF2H1 is significantly linked to MITF abundance and was associated with prognostic melanoma stage, recapitulating the co-expression of MITF and GTF2H1 transcripts in primary and metastatic melanomas. Importantly, GTF2H1 depletion led to a significant reduction in tumor formation in a melanoma xenograft model and was associated with a significantly decreased burden of circulating tumor cells and lung metastases after i.v. injection indicating that TFIIH plays an essential role in the spread of melanoma. These results describe an unanticipated role of MITF in the regulation of NER and the transcription machinery in the melanocytic lineage, which is preserved upon transformation into melanoma. Thereby MITF might coordinately regulate repair and transcription processes optimizing the rapid resumption of transcriptional activity after completion of strand repair, which is vitally important for cellular survival. The very same mechanism may drive the genesis of melanoma and its progression in the context of aberrant transcriptional activity of MITF.

Glycoprotein asporin influences migration and invasion of breast cancer cell lines

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Asporin belongs to small leucine rich proteoglycans family and its crucial characteristic is ability to bind collagens and initiate their mineralization. Asporin plays an important role in normal development of cartilage, bone and teeth. Asporin was initially found to be associated with knee osteoarthritis, however recent research highlights asporin in an association with multiple cancers. This glycoprotein's distinct role in cancer, breast cancer in particular, remains unknown. Based on in silico search, we have found Hs578T breast cancer cell line with asporin expression, that was confirmed by quantitative RT-PCR and western blotting. Human dental pulp stem cells were used for validation of asporin antibodies. Out of multiple testing, we found that asporin can be downregulated by bone morphogenetic protein 4 (BMP4) while upregulation may be facilited by serum-free cultivation or by three dimensional growth in stiff Alvetex® scaffold. Hypoxic conditions had no effect on asporin expression in Hs578T cells. Downregulation by shRNA inhibited invasion of Hs578T through collagen type I matrix while adhesion and spheroid growth were not affected. Invasion of asporin-negative MDA-MB-231 and BT549 breast cancer cells through collagen type I was enhanced by recombinant asporin. In line with other studies, we have confirmed asporin expression by RNA scope in situ hybridization in cancer associated fibroblasts in invasive breast cancer. We have not found any reliable antibody for immunohistochemistry. In conclusion, asporin expression may be regulated by different stimuli from tumor microenvironment, such as 3D culture, starvation or BMP4, which may in turn modulate extracellular matrix and invasion of breast cancer.

Key words: asporin, collagen, breast cancer, invasion, 3D cultivation
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Regulation of the Functional Interface Between Nucleotide Excision Repair and Transcription by MITF Modulates Melanoma Growth

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Survival, proliferation and differentiation of the melanocytic lineage are determined by the MITF. Mechanistically, it is still incompletely understood how MITF is linked to melanomagenesis. However genomic amplification of MITF and SUMOylation-defective MITF germline mutation has been identified in human melanomas. We observed that MITF controls the interface of nucleotide excision repair (NER) and transcription, through transactivation of GTF2H1, a TFIIH core element. Our results show that the NER/TFIIH complex is controlled by MITF in the presence and absence of UVR mediated genotoxic attack resulting in nucleotide repair deficiency and breakdown of global transcription upon MITF depletion. The association between MITF and GTF2H1 transcripts was confirmed utilizing GEOtranscriptome data on a large panel of primary and metastatic melanomas. Moreover, TMA analyses of 140 primary cutaneous melanomas shows that expression of GTF2H1 is significantly linked to MITF abundance and was associated with prognostic melanoma stage, recapitulating the co-expression of MITF and GTF2H1 transcripts in primary and metastatic melanomas. Importantly, GTF2H1 depletion led to a significant reduction in tumor formation in a melanoma xenograft model and was associated with a significantly decreased burden of circulating tumor cells and lung metastases after i.v. injection indicating that TFIIH plays an essential role in the spread of melanoma. These results describe an unanticipated role of MITF in the regulation of NER and the transcription machinery in the melanocytic lineage, which is preserved upon transformation into melanoma. Thereby MITF might coordinately regulate repair and transcription processes optimizing the rapid resumption of transcriptional activity after completion of strand repair, which is vitally important for cellular survival. The very same mechanism may drive the genesis of melanoma and its progression in the context of aberrant transcriptional activity of MITF.

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The Role of Neutrophil Extracellular Traps (NETs) in the Development of Spontaneous Metastasis

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Metastasis is fueled by tumor-associated systemic inflammation. Neutrophils are inflammatory cells that are recruited by various cancer types. Indeed, high neutrophils counts are frequently observed in cancer patients and correlate with poor prognosis. Upon activation, neutrophils release complexes of granular proteins and extracellular DNA, known as neutrophil extracellular traps (NETs), which are essential for control of large pathogens but also seem to be involved in different pathological processes. NETs have been detected in primary human tumors and recent evidence suggests that post-surgical infections promote metastasis via NETs.

In order to dissect the importance of NETs in the metastatic process, we use a model of spontaneous metastasis formation in mice, which occurs after resection of the primary LLC tumor. We observed progressively increased numbers of activated neutrophils in the blood of tumor-bearing mice and detected NETs in primary tumors. Moreover, the number of neutrophils is increased in the lungs of tumor-bearing mice before occurrence of overt metastases and neutrophil depletion before resection reduced the percentage of mice with metastatic disease without affecting the growth of the primary tumor.

Together, these data suggest that neutrophils contribute to initiation and/or progression of metastatic disease and may be involved in building the pre-metastastic niche in target organs such as the lungs. We currently investigate the role of NETs in the metastatic process including formation of the pre-metastatic niche.

Transcriptional Regulation of Prostate Cancer Metabolism

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The current view of cellular transformation and cancer progression supports the notion that cancer cells must undergo a metabolic switch in order to survive and progress in a hostile environment. The cancer metabolism field emerged with the observation of a variety of cancer genes (oncogenes and tumour suppressors) regulating cellular and organism metabolism, in turn linking metabolic disorders to cancer pathogenesis. Metabolic reprogramming in cancer is the integration of a wide array of metabolic routed that need to be coordinatedly altered. Despite the extensive information about the metabolic changes occurring in cancer, we still do not understand which genetic cues are responsible for the coordination of such complex program. Master co-regulators of metabolism orchestrate the modulation of multiple metabolic pathways through transcriptional programs, and hence constitute a probabilistically parsimonious mechanism for general metabolic rewiring. We have approached the study of prostate cancer (PCa) metabolism starting from extensive bioinformatics analysis of large prostate cancer datasets followed by genetic mouse models of prostate cancer, cellular analysis and integrative transcriptomics with state-of-the-art untargeted metabolomics, stable isotope tracing and biochemical assays.We have applied meta-analysis constrains that ensure the selection of relevant candidates contributing to the global metabolic switch, based on two premises: i) they are altered in a significant proportion of prostate cancer studies and ii) they are associated to aggressiveness of the disease. 9 metabolic co-regulators were found altered in the pilot database, while only 3 complied with premise 1. From these 3 co-regulators, only one was associated with aggressiveness. After this medium throughput screen, we validated the relevance of our metabolic regulator through the use of Genetically Engineered Mouse Models of prostate cancer, and surrogate cellular systems. Furthermore, we have used cellular systems to recapitulate the features observed in GEMMs and defined the transcriptional and metabolic means that contribute to prostate cancer progression. In this study we demonstrate that a global metabolic rewiring driven by a anabolic/catabolic switch and the unfavoured use of mitochondrial oxidative programs (i) can be elicited through the alteration of master transcriptional metabolic program, and (ii) opposes the progression and dissemination of prostate cancer.

Preventing Tumour Initiation and Metastasis in Pancreatic Cancer by Targeting the Microenvironment

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The tumour microenvironment (TME) in pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense stroma containing several types of cells which play active roles in tumour formation, progression and invasion. We have previously reported that the TME not only provides structural support for tumour growth, but also provides essential cues to tumour-initiating cancer stem cells (CSC) [1-3]. Tumour associated macrophages (TAMs) are abundant in PDAC TME [4] and have been reported to have several pro-tumourigenic functions in cancer development and metastasis [5] however, the mechanisms by which TAMs promote cancer, particularly in PDAC, are still not completely resolved. Thus, we aimed to further explore how TAMs drive tumour initiation and metastases in PDAC. To identify potential molecular targets, we performed microarray analysis of PDAC cells and Multi-Analyte Profiling analysis of the secretome using simple in vitro models. We also developed 3D-in vitro models based on organotypic cultures for the study of the initial steps of tumour invasion and validation of our targets.

We showed that the interaction between TAMs and PDAC cells drives the up-regulation of key epithelial-mesenchymal transition (EMT) factors and increases the motility and invasive potential of cancer cells. Key genes contributing to this phenotype were identified, such as SerpinB3, known to have pro-migratory, anti-apoptotic and metabolism-related functions. In parallel, secretome analysis revealed a highly pro-inflammatory environment when TAM and PDAC cells were co-cultured. IL-6 and Oncostatin-M have been identified as upstream regulators of SerpinB3, via putative activation of STAT3 signalling.

Our results show that the TAM/PDAC cross-talk is a key regulator of the initial steps of PDAC invasion and EMT. Along with previous findings from our group showing that TAMs regulate cancer stem cell properties, we have now found that TAMs also play a vital role in forming a metastatic niche.

1-Sainz et al. Gut, 2015;
2-Sainz et al. Cancer Research, 2015;
3-Lonardo et al. Cell Stem Cell, 2011;
4-Allavena et al. Crit Rev Oncol Hematol, 2008;
5-Ruffell and Coussens Cancer Cell, 2015

Epigenetic Activation of a Cryptic TBC1D16 Transcript Enhances Melanoma Progression by Targeting EGFR

Miguel Vizoso, Humberto J Ferreira, Paula Lopez-Serra, F Javier Carmona, Anna Martínez-Cardús, Maria Romina Girotti, Alberto Villanueva, Sonia Guil, Catia Moutinho, Julia Liz, Anna Portela, Holger Heyn, Sebastian Moran, August Vidal, Maria Martinez-Iniesta, Jose L Manzano, Maria Teresa Fernandez-Figueras, Elena Elez, Eva Muñoz-Couselo, Rafael Botella-Estrada, Alfonso Berrocal, Fredrik Pontén, Joost van den Oord, William M Gallagher, Dennie T Frederick, Keith T Flaherty, Ultan McDermott, Paul Lorigan, Richard Marais and Manel Estellern

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Metastasis is responsible for most cancer-related deaths, and, among common tumor types, melanoma is one with great potential to metastasize. Here we study the contribution of epigenetic changes to the dissemination process by analyzing the changes that occur at the DNA methylation level between primary cancer cells and metastases. We found a hypomethylation event that reactivates a cryptic transcript of the Rab GTPase activating protein TBC1D16 (TBC1D16-47 kDa; referred to hereafter as TBC1D16-47KD) to be a characteristic feature of the metastatic cascade.

This short isoform of TBC1D16 exacerbates melanoma growth and metastasis both in vitro and in vivo. By combining immunoprecipitation and mass spectrometry, we identified RAB5C as a new TBC1D16 target and showed that it regulates EGFR in melanoma cells. We also found that epigenetic reactivation of TBC1D16-47KD is associated with poor clinical outcome in melanoma, while conferring greater sensitivity to BRAF and MEK inhibitors.

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Metabolic Reprogramming During Initiation of Metastasis Sensitizes Breast Cancer Cells to the Cytotoxic Effects of Fluvastatin

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Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Approximately 20-30% of breast cancers (BrCa) will reoccur after frontline treatment as distant metastases, accounting for 90% of deaths due to BrCa. Initiation of BrCa metastasis occurs when in situ cancer cells undergo epithelial-mesenchymal transition (EMT), acquiring a migratory phenotype that leads to intravasation into the bloodstream. The EMT process is associated with concomitant alterations in glucose metabolism in the cancer cell, including increased dependency on glycolytic activity and increased flux through the pentose phosphate pathway. However, the effects of EMT on other metabolic pathways are unknown. The mevalonate (MVA) pathway is responsible for the biosynthesis of cholesterol and other lipid components important for post-translational modification of proteins. Fluvastatin, a cholesterol-lowering drug, inhibits the rate-limiting enzyme in the MVA pathway. We induced the breast epithelial MCF10A cells to undergo EMT by two approaches: i) by overexpression of oncogenic H- or K-RasG12V, and ii) by overexpression of EMT drivers TWIST or SNAIL. In both cases, we show that EMT sensitizes cells to the cytotoxic effects of fluvastatin, in vitro and *in vivo*. Overexpression of other oncogenes that did not induce EMT, including oncogenic c-MycT58A and several others, did not sensitize MCF10As to fluvastatin, indicating that fluvastatin is synthetically lethal specifically with the EMT process. These results suggest that BrCa cells that have undergone EMT are dependent on the MVA pathway and are uniquely vulnerable to the cytotoxic effects of fluvastatin. These insights could lead to a better understanding of the metabolic dependencies of cells undergoing EMT, and potential repurposing of fluvastatin as a metastatic BrCa prevention agent.

Heterogeneity of Cancer Stem Cell Markers Expression in Breast Cancer Primary Tumors and Lymph Node Metastases – Clinical and Biological Significance

Aleksandra Markiewicz[1], Jolanta Szade[2], Hanna Majewska[2], Tomasz Stokowy[3], Jaroslaw Skokowski[4], Barbara Seroczynska[5], Marzena Welnicka-Jaskiewicz[6], **Anna J Zaczek[1]**

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Cancer stem cells (CSC) are known to drive tumor growth, however detection of CSC markers expressing cells in clinical material might be hampered by tumor heterogeneity. It is also not well understood if heterogeneity of CSC markers in primary tumors (PT) is reestablished in metastases and what is the significance of such heterogeneity. The current work addressed these issues by analyzing levels of CSC markers in different areas PT of the breast and their lymph node metastases (LNM). PT (N=108) and LNM (N=52) were collected from stage I-III breast cancer patients. Protein levels of CSC markers (CD133, ALDH1, CD44, OCT4, NANOG) were analyzed by immunohistochemical staining in up to 5 spatially separated areas of PT and LNM. Level of heterogeneity of each marker expression was calculated with Gini Index (GI), grading samples from non-heterogeneous (GI sore 0) to highly-heterogeneous (GI score 1). Results of IHC analysis were correlated with clinico-pathological parameters, EMT markers expression and dissemination of circulating tumor cells. NANOG, ALDH1 and CD44 were the most abundant CSC markers in PT and LNM. Hierarchical clustering of protein expression revealed presence of 4 clusters in PT – mesenchymal NANOG+ (CD44+/NANOG+), mesenchymal NANOG- (CD44+/NANOG-), epithelial (ALDH1+/NANOG+) and neutral (NANOG+). A trend was observed towards more efficient CTCs dissemination from the mesenchymal NANOG- and neutral clusters (P=0.10). The least expressed markers in PT - CD133 and OCT4 showed one of the highest GI score in PT (0.413 and 0.403), but converted to low GI score in LNM (0.101 and 0.219), respectively; (P=0.0067 for CD133, P=0.09 for OCT4). At the same time average percentage of CD133 and OCT4 positive cells increased in LNM (43.4% and 36.3%) in comparison to PT (19% and 21.2%), respectively. CD133 positive status and its GI heterogeneity index in PT were linked with lack of hormone receptors as well as expression of the EMT marker vimentin (P<0.00001 for all).

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