cnio - CaixaResearch
FRONTIERS
MEETINGS

November 6th - 8th, 2023
Venue: CNIO Auditorium — Madrid • Spain

Metastasis

Organising committee

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

Caroline Dive
Cancer Research UK Manchester Institute, UK

Eva González-Suarez
Spanish National Cancer Research Centre – CNIO, Spain

Héctor Peinado
Spanish National Cancer Research Centre – CNIO, Spain

Manuel Valiente
Spanish National Cancer Research Centre – CNIO, Spain

Speakers

Nicola Aceto
ETH Zurich, Switzerland

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

Arkaitz Carracedo
CICbioGUNE, Vizcaya, Spain

Eduard Batlle
Institute of Biomedical Research (IRB), Spain

Adrienne Boire
Memorial Sloan Kettering Cancer Center, US

Jeremy C. Borniger
Cold Spring Harbor Laboratory, US

Shang Cai
Westlake University, China

Carlos Caldas
Cancer Research UK Cambridge Institute, UK

Caroline Dive
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Mikala Egeblad
Cold Spring Harbor Laboratory, US

Barbara Grüner
West German Cancer Center, Germany

Cyrus Ghajar
Fred Hutchinson Cancer Center, US

Claudia Gravekamp
Albert Einstein College of Medicine, US

Johanna Joyce
Ludwig Institute for Cancer Research, Switzerland

Jean-Christophe Marine
Leuven Center for Cancer Biology, Belgium

Maria Rescigno
Humanitas University, Italy

Erik Sahai
The Francis Crick Institute, UK

Erica Sloan
Monash University, Australia

Humsa Venkatesh
Dana-Farber/Harvard Cancer Center, US

Alana Welm
The University of Utah, US

Frank Winkler
University Hospital Heidelberg and German Cancer Research Center, Germany

Xiang Zhang
Baylor College of Medicine, US

EXCELENCIA SEVERO OCHOA 2012 - 2023
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
Instituto de Salud Carlos III
cnio stop cancer
"la Caixa" Foundation

cnio - CaixaResearch
FRONTIERS
MEETINGS

Madrid 6th – 8th November 2023

Metastasis

#CFM_Metastasis
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Madrid 6th – 8th November 2023

Metastasis

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

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Metastasis

Spanish National Cancer Research Centre (CNIO)
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cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 6th – 8th November 2023

Metastasis

Summary

cnio stop cancer

Madrid 6th – 8th November 2023

Metastasis

Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

cnio stop cancer

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 6th – 8th November 2023

Metastasis

Organisers and Speakers

cnio stop cancer

Madrid 6th – 8th November 2023

Metastasis

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid.

Organisers & Speakers

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

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Héctor Peinado
Spanish National Cancer Research Centre – CNIO, Spain

Manuel Valiente
Spanish National Cancer Research Centre – CNIO, Spain

CNIO - CaixaResearch Frontiers Meeting

Speakers

Nicola Aceto
ETH Zurich, Switzerland

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

Arkaitz Carracedo
CICbioGUNE, Vizcaya, Spain

Eduard Batlle
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FRONTIERS
MEETINGS

Madrid 6th – 8th November 2023

Metastasis

Programme

cnio stop cancer

Monday November 6th, 2023

08.30 – 09.00 Registration

09.00 – 09.15 Welcome

Session 1.	**Modelling evolution of metastasis** **Chair: Héctor Peinado**
09.15 – 09.45	*How do dormant tumor cells evade immune recognition, and what can we do about it?* ***Cyrus Ghajar*** *Fred Hutchinson Cancer Center, US*
09.45 – 10.00	***[Short talk]:*** *Deconstructing immunity of breast cancer micrometastasis* ***Toni Celiá*** *Hospital del Mar Research Institute, Spain*
10.00 – 10.15	***[Short talk]:*** *RET overexpression leads to increased brain metastatic competency in luminal breast cancer* ***Petra Jagušt*** *RCSI, University of Medicine and Health Sciences, Ireland*
10.15 – 10.30	***[Short talk]:*** *Inactivation of p53 drives breast cancer brain metastasis by altering fatty acid metabolism* ***Uri Ben-David*** *Tel Aviv University, Israel*

Monday November 6th, 2023

10.30 - 11.00 Coffee break & poster session (social room)

11.00 – 11.30	*Why liver metastases undermine immunotherapy* ***Erik Sahai*** *The Francis Crick Institute, UK*
11.30 – 12.00	*Studying melanoma evolution one cell at the time* ***Jean-Christophe Marine*** *Leuven Center for Cancer Biology, Belgium*
12.00 – 12.15	***[Short talk]:*** *Metastatic colonization requires a proliferative pause linked to vascular co-option* ***Pedro García Gómez*** *Spanish National Cancer Research Centre, Spain*

12.15 - 13.45 Lunch break (cafeteria)

Monday November 6th, 2023

Session 2.	Non-Genetic adaptation in metastasis (metabolism, epigenetics and stress) Chair: María Caffarel
13.45 – 14.15	*Metabolic reprogramming at the core of prostate cancer progression and metastasis* ***Barbara Grüner*** *West German Cancer Center, Germany*
14.15 – 14.45	*Metabolic reprogramming at the core of prostate cancer progression and metastasis* ***Arkaitz Carracedo*** *CICbioGUNE, Vizcaya, Spain*
14.45 – 15.00	***[Short talk]:*** *Impact of obesity in breast cancer pre-metastatic niche formation* ***Marta Hergueta*** *Spanish National Cancer Research Centre, Spain*
15.00 – 15.30	*The bone marrow niches as targets and sources of metastases* ***Xiang H-F Zhang*** *Baylor College of Medicine, US*

15.30 - 16.00 Coffee break & poster session (social room)

Session 3.	Microorganismal influence on metastasis Chair: Caroline Dive
16.00 – 16.30	*The microbiota in cancer progression and metastasis* ***Maria Rescigno*** *Humanitas University, Italy*
16.30 – 17.00	*Tumor resident bacteria promote metastatic colonization and immune evasion in breast cancer* ***Shang Cai*** *Westlake University, China*
17.00 – 17.30	*Tumor-targeted delivery of a neoantigen surrogate by listeria reduces pancreatic cancer* ***Claudia Gravekam*** *Albert Einstein College of Medicine, US*

17.30 – 19.30 Poster session with light dinner (social room)

Tuesday November 7th, 2023

Session 4.	Anti-metastasis therapies and clinical efforts Chair: Eva González
09.00 – 09.30	*Capturing and exploiting cancer cell metabolic plasticity in the leptomeninges* ***Adrienne Boire*** *Memorial Sloan Kettering Cancer Center, US*
09.30 – 10.00	*Stem cells, immune evasion and metastasis in colorectal cancer* ***Eduard Batlle*** *Institute of Biomedical Research (IRB), Spain*
10.00 – 10.15	***[Short talk]:*** *Immunotherapy-induced blood brain barrier (BBB) opening: Implications for combination drug scheduling* ***Abhilash Nitin Deo*** *Technion - Israel institute of Technology, Israel*
10.15 – 10.30	***[Short talk]:*** *TIMP1 mediates astrocyte-dependent local immunosuppression in brain metastasis acting on infiltrating CD8+ T cells* ***Neibla Priego*** *Spanish National Cancer Research Centre, Spain*

10.30 - 11.15 Group picture (main door) coffee break & poster session (social room)

11.15 – 11.45	*Tackling metastases as cellular ecosystems* ***Carlos Caldas*** *Cancer Research UK Cambridge Institute, UK*
11.45 – 12.15	*Immune-mediated regulation of breast cancer metastatic outgrowth* ***Alana Welm*** *The University of Utah, US*

12.15 - 13.45 Lunch break (cafeteria)

Tuesday November 7th, 2023

Session 5.	Influence of microenvironment in metastasis Chair: Julio Aguirre-Ghiso

13.45 – 14.15 *NETworking in cancer: bidirectional interactions between cancer and neutrophil extracellular traps (NETs) as regulators of metastasis*
Mikala Egeblad
Cold Spring Harbor Laboratory, US

14.15 – 14.30 ***[Short talk]:*** *Macrophage-fibroblast JAK/STAT dependent crosstalk promotes liver metastatic outgrowth in pancreatic cancer*
Meirion Raymant
University of Liverpool, UK

14.30 – 14.45 ***[Short talk]:*** *In vivo screening of tumor-hepatocyte interactions identifies Plexin B2 as a gatekeeper of liver metastasis*
Constanza Borrelli
ETH Zurich, Switzerland

14:45 - 16:45 Coffe break & poster session (social room)

16.45 – 17.00 ***[Short talk]:*** *RANK pathway inhibition impairs immunosupression in macrophages enhancing the anti-tumour response*
Alexandra Barranco
Spanish National Cancer Research Centre, Spain

17.00 – 17.15 ***[Short talk]:*** *MAF amplification licenses Estrogen Receptor α to drive breast cancer bone metastasis*
Roger Gomis
Institute for Research in Biomedicine, Spain

17.15 – 17.45 *Microenvironmental Regulation of Metastasis*
Johanna Joyce
Ludwig Institute for Cancer Research, Switzerland

19.30 Social event

Wednesday November 8th, 2023

Session 6.	CTCS/ DTC/ CTDNA: New technologies and implications Chair: Paloma Bragado

09.00 – 09.30 *Circulating Tumour Cells and Lung Cancer Metastasis*
Caroline Dive
Cancer Research UK Cambridge Institute, UK

09.30 – 10.00 *Circulating tumor cell clusters*
Nicola Aceto
Institute of Molecular Health Sciences (IMHS), Switzerland

10.00 – 10.15 ***[Short talk]:*** *Circulation-on-a-Chip: Cell Survival Under Pro-Apoptotic Mechanical Cues in Metastasis*
Marc Rico-Pasto
University of Barcelona, Spain

10.15 – 10.45 *Age-related clonal hematopoiesis drives awakening of dormant breast cancer cells in the lung*
Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

10.45-11.15 Coffee break & poster session (social room)

Wednesday November 8th, 2023

Session 7.	Neurobiology of metastasis Chair: Manuel Valiente
11.15 – 11.45	*Targeting neural signalling to prevent relapse after cancer treatment* ***Erica Sloan*** *Monash University, Australia*
11.45 – 12.15	*The Neurobiology of Brain Metastasis* ***Frank Winkler*** *University Hospital Heidelberg and DKFZ, Germany*
12.15 – 12.30	*[Short talk]: Gap-Junction mediated calcium oscillations drive melanoma brain metastasis progression* ***Nils Hebach*** *U. Hospital Heidelberg / German Cancer Research Institute, Germany*
12.30 – 13.00	***Humsa Venkatesh*** *Dana-Farber/Harvard Cancer Center, US*
13.00 – 13.30	*Neural basis for blunted circadian glucocorticoid rhythms in breast cancer* ***Jeremy Borniger*** *Cold Spring Harbor Laboratory, US*
13.30 - 13.45	*Best posters and short talks prizes*

13.45 Closing remarks

Lunch at the cafeteria

Attending editors

Gemma Alderton *(Science)*

Elizabeth S McKenna *(Cancer Discovery)*

Ivayla Ivanova *(Developmental Cell)*

Co-organizers

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

Caroline Dive
Cancer Research UK Manchester Institute, UK

Eva González-Suarez
Spanish National Cancer Research Centre – CNIO, Spain

Héctor Peinado
Spanish National Cancer Research Centre – CNIO, Spain

Manuel Valiente
Spanish National Cancer Research Centre – CNIO, Spain

Schedule may change due to unforeseen circumstances.

cnïo - CaixaResearch
FRONTIERS
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Metastasis

Monday November 6th 2023

Session #1
Modelling evolution of metastasis

Chairperson: ***Héctor Peinado***

cnïo *stop cancer*

How do dormant tumor cells evade immune recognition, and what can we do about it?

Cyrus Ghajar
Translational Research Program
Fred Hutchinson Cancer Center
Washington, US

From the moment of initiation to the time they are detected, millions of tumor cells disseminate from their site of origin. A very small fraction of these cells might eventually evolve into metastases that compromise function of vital organs and cause lethality. My laboratory studies mechanisms by which we can prevent this process from occurring, primarily by studying the successful template laid out by the incredibly large fraction of tumor cells that are never able to successfully execute the last step of this program. Through intravital imaging experiments, we established that escaping from dormancy is the rate-limiting phase of metastases, and thus we sought ways we could eradicate cells while they are still in a dormant state. One lens we've applied is an immunological one; specifically, to understand how dormant tumor cells evade immune surveillance. I will describe the unique approach we took to answer this question, and its surprising conclusion. We are now testing the implications of our findings in people, and this is where things really get exciting because we endeavor to invent and/or adopt computational approaches to find the answers.

Metastasis

SHORT TALK

Deconstructing immunity of breast cancer micrometastasis

Toni Celià-Terrassa
Hospital del Mar Research Institute, Spain

During metastasis only a minority of disseminated tumor cells (DTCs) with advantageous properties survive and overcome the hostile conditions of distant microenvironments. One of the most important barriers of metastasis to surpass is the immune system. In this regard, how early tumor-immune interactions during metastatic seeding impact the transition to macrometastasis is not completely understood. Using single cell RNA-seq of CD45+ cells of breast cancer (BC) micrometastasis in mouse models, we observed immune remodeling and immunosuppressive engagement at the very early moments of metastasis. In particular, we found an enrichment of IL-17 gdT-cells which are immunosuppressive immune cells leading a permissive immunity and consequent metastatic outbreak. In order to understand how DTCs execute this microenvironmental influence, we have explored by RNA-seq the transcriptomic profiles of BC metastatic cells selected by immune pressure in different organs after intracardiac transplantation.
Unexpectedly, we have found TIM3 – a receptor typically expressed in immune cells – among the top upregulated genes in metastatic immunoedited cells in different BC mouse models, in particular associated to EMT-like phenotypes. Next, we verified that the immune pressure of distant organs strongly selected TIM3+ tumor cells specifically during micrometastasis. Mechanistically, the loss of TIM3 reduced b-Catenin signaling and the presence of gdT-cells in micrometastasis, thus impairing metastasis in mouse models. Using clinical samples from the ConvertHER trial, we confirmed the enrichment of TIM3+ cells in BC metastasis. Moreover, BC patients with TIM3+ cells concur with poor overall survival prognosis. In preclinical settings, the anti-TIM3 blockade antibody treatment in mice showed a significant delay of overall metastasis. Therefore, these results encourage the potential clinical application of TIM3 blockade and time-tailored therapies targeting metastaticinitiating cells.

SHORT TALK

RET overexpression leads to increased brain metastatic competency in luminal breast cancer

Petra Jagušt
RCSI, University of Medicine and Health Sciences, Ireland

Breast cancer represents a frequent source of brain metastasis (BCBM), with rising incidence. A better understanding of the mechanisms of how breast cancer cells consistently and successfully colonize the brain could lead to therapies that are more effective in managing this advanced disease. In the present study, we explored the role of the tyrosine kinase receptor, RET, in promoting breast cancer brain metastasis. Using clinical datasets, mouse and patient-derived brain metastatic models, we found that RET is significantly enriched in brain metastasis originating from estrogen receptor-positive breast cancer where it plays a key role in promoting cancer cell adhesion, survival, and outgrowth in the brain. Moreover, cells with aberrant RET expression were also found to have a higher competency for brain metastasis *in vivo* in a patient-derived model, revealing the clinical significance of RET in BCBM. Mechanistically, RET overexpression was found to enhance the activation of gene programs involved in cell adhesion, requiring epidermal growth factor receptor (EGFR) cooperation to deliver a pro-brain metastatic phenotype. Finally, the relevance of RET overexpression was assessed in patient-derived brain metastatic tumour explants, organoid models and brain organotypic cultures where RET inhibition demonstrated functional efficacy. Our findings provide novel insights into the role of RET in controlling the colonization and expansion of brain metastasis, which could inform approaches to manipulate the RET-signalling axis as a treatment approach for breast cancer brain metastasis.

Other Information:
Utilising a substantial cohort of primary breast cancer tissues (N=820) together with *in vivo*, *ex vivo* and *in vitro* preclinical models of BCBM, we investigated the functionality of RET overexpression in brain metastasis. We demonstrate RET's clinical relevance in extended transcriptome data from ER+ve brain metastatic patients (N=23 pairs of matched primary and brain metastasis) and its therapeutic utility in patient-derived brain metastatic explants and organoids (N= 6 BCBM models).

SHORT TALK

Inactivation of p53 drives breast cancer brain metastasis by altering fatty acid metabolism

Uri Ben-David
Tel Aviv University, Israel

Brain metastasis is a dire prognosis across cancer types. It is largely unknown why some tumors metastasize to the brain whereas others do not. We analyzed genomic and transcriptional data from clinical samples of breast cancer brain metastases (BCBM) and found that almost all (>98%) of them carried p53 inactivating genetic alterations through mutations, copy-number loss, or both. Importantly, p53 pathway activity was already perturbed in primary tumors giving rise to BCBM, often by loss of the entire 17p chromosome-arm. Experimentally, p53 knockout in mouse and human breast cancer cell lines was sufficient to drastically increase BCBM formation and growth *in vivo*, providing a causal link between p53 inactivation and brain tropism. Mechanistically, p53-deficient breast cancer cells exhibited altered lipid metabolism, and in particular increased fatty acid synthesis (FAS) and uptake, which are characteristic of brain-metastasizing cancer cells. FAS was further promoted by astrocytes in a p53-dependent manner, as astrocyte conditioned-medium increased FASN and SCD1 expression and activity, and preferentially improved the survival, proliferation and migration of p53- deficient cells. Consequently, p53-deficient cells were more sensitive than p53- competent cells to FAS inhibitors, in isogenic cell cultures, in tumor-derived spheroids, and across dozens of breast cancer cell lines. Lastly, a significant association was observed between p53 inactivation, astrocyte infiltration and SCD1 expression in two independent cohorts of human BCBM samples, demonstrating the clinical relevance of our findings. In sum, our study identifies p53 inactivation as a driver of BCBM and potentially of brain metastasis in general; suggests a p53- dependent effect of astrocytes on breast cancer cell behavior; and reveals FAS as an underlying, therapeutically-targetable molecular mechanism.

Why liver metastases undermine immunotherapy

Erik Sahai
The Francis Crick Institute
London - UK

Erik Sahai PhD is the head of the Tumour Cell Biology laboratory at the Francis Crick Institute in London. 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). Following this training, Erik set up his own group at the Cancer Research UK London Research Institute in 2004 and then transferred to the Francis Crick Institute in 2015. His research is focused on the spread of cancer through the body and responses to cancer therapy. In particular, his group is interested in stromal fibroblasts and their interplay with both tumour and immune cells. 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.

Studying melanoma evolution one cell at the time

Jean-Christophe Marine
Professor at KU Leuven
Director of the VIB center for Cancer Biology
Leuven - Belgium

Although melanoma is notorious for its high degree of heterogeneity and plasticity, the origin and magnitude of cell state diversity remains poorly understood. Equally, it is not known whether melanoma growth and metastatic dissemination are supported by overlapping or distinct melanoma subpopulations. By combining mouse genetics, unbiased lineage tracing and quantitative modelling, single-cell and spatial transcriptomics, we provide evidence of a hierarchical model of tumour growth that mirrors the cellular and molecular logic underlying embryonic neural crest cell fate specification and differentiation. Our findings indicate that tumorigenic competence is associated with a spatially localized perivascular niche environment, a phenotype acquired through a NOTCH3-dependent intercellular communication pathway established by endothelial cells. Consistent with a model in which only a fraction of melanoma cells is fated to fuel growth, temporal single-cell tracing of a population of melanoma cells harbouring a mesenchymal-like state revealed that these cells do not contribute to primary tumour growth but, instead, constitutes a pool of metastatic-initiating cells that can switch cell identity while disseminating to secondary organs. Our data provide a spatially and temporally resolved map of the diversity and trajectories of cancer cell states within the evolving melanoma ecosystem and suggest that the ability to support growth and metastasis are limited to distinct pools of melanoma cells. The observation that these phenotypic competencies can be dynamically acquired upon exposure to specific niche signals warrant the development of therapeutic strategies that interfere with the cancer cell reprogramming activity of such microenvironmental cues

SHORT TALK

Metastatic colonization requires a proliferative pause linked to vascular co-option

Pedro García Gómez
Spanish National Cancer Research Centre CNIO, Spain.

The development of preventive strategies against muti-organ metastases requires common mechanisms that could be targeted as well as the identification of windows of opportunity representing clinically-relevant scenarios that can be exploited. The interaction of extravasated metastatic cells with the pre-existing vasculature of the organ being colonized, known as vascular co-option, has been previously reported as a key process for metastasis initiation. Here we show that vascular co-opting metastatic cells from aggressive brain metastasis models enter into a cellular state characterized by a major reduction of their proliferation (80%).
This temporary cell behavior effectively counteracts multiple sources of stress (i.e., DNA damage) affecting metastasis-initiating cells enabling them to survive and subsequently resume their aggressive proliferation to colonize the target organ.
The proliferative pause is driven by the MYC antagonist MXD4 and involves a transient molecular switch in metastasis-initiating cells that can be targeted.
Pharmacologic approaches in clinically relevant models where vascular co-option and the proliferative pause are present, including relapse post-surgery and spontaneous models of brain metastasis, validate this preventive therapeutic strategy. The translational potential of our findings was further confirmed on human micrometastases identified in autopsies as well as by targeting the invasive fronts of alive surgically resected metastases. Thus, our results report a novel cellular step within the metastatic cascade during the initial stages of organ colonization in aggressive models of metastasis. The proliferative pause might have broader potential implications in the biology and the clinical presentation of, at least, brain metastasis as it might contribute to the increased genomic instability and the divergent evolution from the primary tumor. Most importantly, the vulnerabilities found in the proliferative pause and its compatibility to clinically relevant situations (i.e., relapse post-surgery) represent a window of opportunity to develop clinical trials to prevent metastases.

Madrid 6th – 8th November 2023

Metastasis

Monday November 6th 2023

Session #2
Non-genetic adaptation in metastasis (metabolism, epigenetics and stress)

Chairperson: ***María Caffarel***

Nutrient dependencies of metastasis formation

Barbara Grüner
West German Cancer Center, Germany

Metabolic reprogramming at the core of prostate cancer progression and metastasis

Arkaitz Carracedo
CICbioGUNE, Vizcaya, Spain

SHORT TALK

Impact of obesity in breast cancer pre-metastatic niche formation

Marta Hergueta
Spanish National Cancer Research Centre, Spain

Metastasis in cancer is a complex process with unfavorable outcomes. Obesity is associated with adverse prognosis in breast cancer (BC). Beyond its role in primary tumor development, obesity triggers chronic inflammation and hypercoagulability, affecting metastasis. It is known that platelets contribute to the survival and spread of circulating tumor cells, however the link between obesity, coagulation and premetastatic niche (PMN) formation is still not clear.

We found that obesity creates unique microenvironments in distant organs, called "obese PMNs" (Ob-PMNs). Mice on a high-fat diet (HFD) showed enhanced platelet aggregation, increased P-Selectin expression, and lung vascular permeability, activating genes related to adhesion, extracellular matrix, and coagulation. We observed fibronectin (FN) overexpression in lung Ob-PMNs and platelets of HFDfed mice.

Intravital microscopy demonstrated that HFD-fed mice had increased lung metastases, and enhanced tumor cell adhesion to platelets and endothelial cells (ECs). Mechanistically, platelets and ECs from HFD-fed mice exhibited activation of pathways linked to increased platelet adhesion and EC permeability such as Src and Talin. Switching mice from an HFD to a normal diet reduced weight and normalized FN levels in platelets. This dietary shift also resulted in reduced tumor cell homing and improved vascular permeability. Furthermore, depleting platelets in HFD-fed mice using anti-GPIbα antibodies reduced tumor cell homing and metastasis, underscoring a role of platelets in BC metastasis and Ob-PMN formation. Analysis in TNBC patients revealed an inverse correlation between body mass index (BMI) and activated partial thromboplastin time (aPTT), with shorter aPTT linked to accelerated disease relapse. In conclusion, we propose that obesity-induced platelet activation create a Ob- PMNs easing BC metastasis. Targeting platelets or dietary intervention may hold therapeutic potential in BC, especially in obese individuals.

The bone marrow niches as targets and sources of metastases

Xiang H.-F. Zhang

Professor of Molecular and Cellular Biology The William T. Butler, M.D.
Endowed Chair for Distinguished Faculty Interim Director,
Lester and Sue Smith Breast Center McNair Scholar
Baylor College of Medicine - Houston - US

Many solid cancers metastasize to the bone and bone marrow (BM). This process may occur even before diagnosis of primary tumors. The cellular fates and metastatic progression of DTCs are determined by complicated interactions between cancer cells and bone marrow niches. Not surprisingly, these niches also play important roles in normal biology, including homeostasis and turnover of skeletal and hematopoiesis systems. Our previous research on metastatic niches leveraged on existing knowledge of bone and bone marrow microenvironment. In this lecture, I will introduce an unbiased approach that can facilitate single-cell molecular and cellular profiling of microenvironment cells that are in direct contact with metastatic cells. Using this approach, we identified key constituents of the metastatic niches in bone and other organs. Surprisingly, we discovered that the estrogen receptor signaling in macrophages plays a pivotal role in bone metastatic colonization even in non-breast cancer models and male mice, which implicates a much broader application of endocrine therapies in treatment of bone metastasis.

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Metastasis

Monday November 6th 2023

Session #3
Microorganismal influence on metastasis

Chairperson: ***Caroline Dive***

Microbiota in cancer progression and metastasis

Maria Rescigno
Vice Rector and delegate for research
Humanitas University, Pieve Emanuele
Milan – Italy

Metastatic Colorectal Cancer (mCRC) is the third leading cause of cancer-related mortality. After surgical resection of the primary tumor, from 30% to 40% of colorectal cancer patients develop distant metastases in five years. Spreading of neoplastic cells from CRC to regional LN correlates with poor patient outcome and it has been used as a prognostic marker of recurrence for treatment assignment. Detection of neoplastic cells in regional lymph nodes (LN) of colorectal cancer (CRC) patients is associated with distant recurrence, but lymphadenectomy does not increase CRC patients' survival. This suggests that the lymphatics are not the only way of tumor dissemination. Indeed, metastatic cells could also disseminate via the systemic blood circulation but the gut vascular barrier (GVB) should be eluded. The GVB inhibits bacteria dissemination from the gut to the liver, via the portal circulation. We found that the GVB is involved in the hematogenous dissemination of metastatic tumor cells and this is dependent on the microbiota. Changes in microbiota composition during tumor development can contribute on one hand to lose protective microbiota and on the other to expand protumorigenic members capable of favoring tumor development and metastasis formation by modifying the GVB. In a retrospective analysis of two cohorts of CRC patients we associated increased detection of PV-1, a marker of GVB disruption, to metachronous distant metastases. We demonstrate that PV-1 is an independent new prognostic biomarker of CRC distant recurrence ($P<0.0001$). Moreover, we provide evidence that GVB derangement is associated with bacteria dissemination in metastatic livers. In two preclinical models of CRC, we show that bacteria disseminate before cancer cell seeding and boost the formation of a pre-metastatic niche at distant sites, which is then able to favor the recruitment of metastatic cells. Thus, we demonstrate that vascular impairment, bacteria and tumor metastatization are linked processes and PV-1 is a new promising prognostic marker for distant recurrence. We will discuss this new mechanism of tumor cell dissemination and the players involved.

Tumor resident bacteria promote metastatic colonization and immune evasion in breast cancer

Shang Cai

Shang Cai Ph.D. Assistant Professor of Westlake University
Westlake University, China

The intratumor microbiota have recently been recognized as an integral component in both murine and human cancers, and play important roles in cancer metastasis, drug response, as well as cancer patient prognosis. We previously showed that intratumor bacteria predominantly reside in the cytosol of breast cancer cells, and regulate mechanical stress response pathways to promote the survival of cancer cells during metastatic colonization. However, after colonization, how cancer cells with intracellular bacteria adapt to the distal organ microenvironment remains unclear. In this study with PyMT breast cancer model, through luciferase tracing analysis of the metastatic cells, we found that elimination of intracellular bacteria impaired metastasis development after cancer cells settled in the lung in an immune dependent manner. Further immune system analysis showed that neutrophils in the metastatic niche are the major cellular component that initially responded to the bacteria invaded cancer cells. Single cell RNAseq revealed that intracellular bacteria induced neutrophils to a pro-tumor N2 state (PMN-MDSC) and suppressed T cell activation, while extracellular bacteria drove neutrophils to an anti-tumor N1 state. Mechanistically, the intracellular bacteria activated both NFkb and STING pathway in the host cells to promote secretion of immune suppressive cytokines and dictate the PMN-MDSC fate. Elimination of the intracellular bacteria can efficiently unleash the immune suppression in the metastatic foci and restrict the metastasis development. Therefore, the intratumor bacteria plays a key role in shaping the immune metastatic niche, and harnessing extracellular bacteria and intracellular bacteria may have distinct consequences in the immunotherapy.

Tumor-targeted delivery of a neoantigen surrogate by listeria reduces pancreatic cancer

Claudia Gravekamp, PhD

Claudia Gravekamp, PhD, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York, USA

Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic disease. Tumors are poorly immunogenic due to its lack of effective neoantigens and strong immune suppression, preventing T cell activation in the tumor microenvironment. Here, we present a novel microbial-based immunotherapeutic treatment for selective delivery of an immunogenic tetanus toxoid protein ($TT^{856-1313}$) into tumor cells as a neoantigen surrogate by attenuated *Listeria monocytogenes*. This treatment reactivates pre-existing TT-specific memory T cells generated during childhood to kill infected tumor cells. Treatment of $Kras^{G12D/+}$; LSL-$Trp53^{R172H/+}$ (KPC) mice with Listeria-TT resulted in TT accumulation inside tumor cells, attraction of TT-specific memory CD4 T cells to the tumor microenvironment and the production of perforin and granzyme B in tumors. Low doses of gemcitabine (GEM) increased the immune effects of Listeria-TT, turning immunologically cold into hot tumors in mice. *In vivo* depletion of T cells from Listeria-TT+GEM-treated mice demonstrated a CD4 T cell-mediated reduction in tumor burden. CD4 T cells from TT-vaccinated mice were able to kill TT-expressing Panc-02 tumor cells *in vitro*. Whereas CD4 T cells efficiently infiltrated the KPC tumors, CD8 T cells did not. Listeria-TT+GEM treatment of KPC mice with advanced PDAC reduced tumor burden by 80% and metastases by 87% after treatment, and increased survival by 40% compared to non-treated mice. This treatment regimen is not only effective against pancreatic cancer. Very recently, we also showed that Listeria-TT+GEM was highly effective against metastatic ovarian cancer. These results support the use of Listeria-delivered recall antigens as a powerful alternative to neoantigen-mediated cancer immunotherapy.

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Metastasis

Tuesday November 7th 2023

Session #4
Anti-metastasis therapies and clinical efforts

Chairperson: ***Eva González***

cnio stop cancer

Capturing and exploiting cancer cell metabolic plasticity in the leptomeninges

Adrienne Boire
Memorial Sloan Kettering Cancer Center, US

Metastatic cancer cells confront microenvironments unlike those of the primary tumor. Unlike their constrained non-transformed counterparts, cancer cells enjoy considerable epigenetic freedom. In doing so, they employ programs from a variety of physiologic contexts to "solve" the problem of living within these starkly foreign environments. The leptomeninges, the fluid-filled interface between the systemic circulation and the central nervous system represents a nutritionally sparse, anatomically isolated location posing substantial constraints on cancer cell growth. To investigate the mechanism(s) whereby cancer cells might overcome these nutritional constraints, we leveraged our mouse models of site-specific metastasis, generated by iterative *in vivo* selection. ATAC-Seq and RNASeq of leptomeningeal-metastatic (LeptoM) cells collected from the leptomeninges compared with those from the orthotopic primary site revealed a unique leptomeningeal chromatin state, and enrichment of genes downstream of LXR/RXR response elements, including de novo fatty acid synthetic pathways. Indeed, we find that retinol levels are elevated in the CSF of patients harboring leptomeningeal metastasis, and retinol biosynthetic pathway genes expressed within the leptomeningeal fibroblasts. In addition, scRNA Seq of cancer cells collected from the CSF of LM patients revealed elevated expression of retinol uptake and processing genes. *In vitro*, RXR agonists induce expression of FASN and ACACA, rate-limiting enzymes of fatty acid biosynthesis. *In vivo*, genetic knock out of cancer cell expression of retinol uptake (STRA6), fatty acid biosynthesis (FASN), LXR (NR1H3, NRiH2), or RXR signaling (RARA, RARB), inhibits LeptoM cancer cell growth in the leptomeninges, but does not alter growth of these cells elsewhere. Together, these results support a model whereby leptomeningeal-generated RA induces cancer cells to carry out de novo lipid biosynthesis to support their growth, and suggests pharmacological targets to arrest this growth.

Stem cells, immune evasion and metastasis in colorectal cancer

Eduard Batlle

ICREA & Cancer Science Program, Institute for Research in Biomedicine (IRB Barcelona).
The Barcelona Institute of Science and Technology (BIST)
Barcelona, Spain

Most colorectal cancer (CRC) patients die as a result of metastasis. Neither conventional chemotherapy nor current targeted therapies offer significant benefits once the disease has spread to distant organs. Furthermore, current CRC staging based on histopathology and imaging has a limited ability to predict the evolution of the disease. We discovered that vast majority of genes that distinguish poor prognosis CRC subtypes are expressed by cancer-associated fibroblasts. We showed that metastasis relies on a tumor cell non-autonomous program driven by TGF-beta in the tumor microenvironment, which drives T cell exclusion and immune evasion. Here I will discuss our latest data on how stromal cells help disseminated tumor cells evade the attack of the immune system and the design of new therapeutic strategies based on targeting the tumor microenvironment.

SHORT TALK

Immunotherapy-induced blood brain barrier (BBB) opening: Implications for combination drug scheduling

Abhilash Nitin Deo
Technion - Israel institute of Technology, Israel

Brain metastasis (BM) is an impediment in the clinical management of several solid tumors. Although immune checkpoint inhibitors (ICIs) offer a significant therapeutic benefit to patients with BM, the intra-cranial response is often heterogenous. Recently, studies have documented the emergence and progression of BM in melanoma and non-small cell lung carcinoma (NSCLC) patients post-ICI treatment. However, the underlying molecular mechanism(s) remain unknown. We used a breast cancer mouse model of spontaneous response to anti-PD1 to map the pre-metastatic niche in the brain after anti-PD1 treatment. High throughput mass cytometry (CyTOF) analysis revealed increased infiltration of myeloid cells in anti-PD1-treated brains as compared to IgG-treated controls, indicating an immunosuppressive microenvironment. Moreover, single cell RNA sequencing and gene enrichment analyses demonstrated downregulation of Wnt signalling in endothelial cells of anti-PD1 treated brains, indicating compromised blood brain barrier (BBB) function. Indeed, mice pre-conditioned with anti-PD1 displayed BBB disruption and accelerated experimental BM. This effect was mediated by plasmaborne DKK1, a Wnt signalling inhibitor, induced in response to anti-PD1 treatment.

Plasma DKK1 inhibition restored BBB function and ameliorated the progression of experimental BM after anti-PD1 treatment in our pre-clinical models. Clinically, longitudinal increases in plasma DKK1 levels were correlated with incidence of BM in NSCLC patients undergoing immunotherapy. To further gain clinical insights into the immunotherapy-based drug combination, we found that the induction with anti- PD1 potentiated the intracranial response to cisplatin in mice with BM from immunotherapy-resistant lung cancer compared to cisplatin monotherapy. Thus, anti-PD1-induced BBB opening may be a potential strategy to increase the efficacy of BBB-impermeable chemotherapy, especially for management of ICI nonresponding patients with BM.

Awarded

SHORT TALK

TIMP1 mediates astrocyte-dependent local immunosuppression in brain metastasis acting on infiltrating CD8+ T cells

Neibla Priego
Spanish National Cancer Research Centre (CNIO), Spain

Recently, immune checkpoint blockade antibodies (ICB) have shown clinical benefits mainly when applied to asymptomatic brain metastasis patients. However, variability is broad and responses to this therapy drop considerably when treating clinically relevant disease. Although potentially involved in the lack of response to immunotherapy, corticoids do not seem to fully explain this situation. Thus, it is currently unknown how to effectively target symptomatic brain metastases with immunotherapy.

We previously reported a clinically relevant protumoral program driven by STAT3 activation in a subpopulation of reactive astrocytes during advanced stages of the disease. By further exploiting astrocyte heterogeneity, we have found a potential strategy to improve the percentage of responders to immunotherapy in symptomatic brain metastasis. Specifically, we have developed a genetic approach to report a novel immunosuppressive mechanism initiated by astrocyte-secreted TIMP1 that signals on CD8+ T cells by binding to the receptor CD63. Based on these findings we have developed a combined immunotherapy that boost the systemic activation of T cells with ICB while simultaneously blocks TIMP1-dependent immunosuppression locally. This pharmacological strategy increased the limited benefit of ICB in various experimental brain metastasis models. Furthermore, the detection of TIMP1 in the cerebrospinal fluid (CSF) of patients with brain metastases suggests a biomarker to personalized this therapeutic approach. As a proof-of-concept we tested the benefit of targeting the TIMP-1-dependent local immunosuppression in alive human brain metastases using Patient Derived Organotypic Cultures (PDOC) established from fresh neurosurgeries, which confirmed the relevance of our findings.

Overall, our study confirms the potential of addressing functional heterogeneity within the microenvironment (i.e., TIMP1+ STAT3+ reactive astrocytes) to improve the efficacy of therapies against metastasis.

Metastasis

Tackling metastases as cellular ecosystems

Carlos Caldas
Cancer Research UK Cambridge Institute

Immune-mediated regulation of breast cancer metastatic outgrowth

Alana Welm
The University of Utah
Utah, US

We are investigating a novel “immune checkpoint” that is different from classic immune checkpoints but still serves to restrict T cell activity in metastatic breast tumors in mouse models. Blocking Ron kinase activity works remarkably well to boost anti-tumor immune responses and eliminate metastases. We found that blocking a particular isoform of Ron kinase robustly expands a stem-like CD4+ T helper cell population that leads to swarming of metastatic lesions by T cells, and continues to fuel the immune response when, normally, exhaustion would occur. Ron kinase inhibitors have been in clinical trials and are well-tolerated, and our work indicates we can successfully inhibit Ron function in advanced cancer patients at a tolerable dose. This finding paves the way for a new immunotherapy strategy in metastatic breast cancer. I will also briefly discuss our collection of patient derived models for metastatic breast cancer, and their availability to the community for discovery science and preclinical research.

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Session #5
Influence of microenvironment in metastasis

Chairperson: ***Julio Aguirre-Ghiso***

Metastasis

NETworking in cancer: bidirectional interactions between cancer and neutrophil extracellular traps (NETs) as regulators of metastasis

Mikala Egeblad
Cold Spring Harbor Laboratory, US

Chronic stress is associated with increased risk of metastasis and poor survival in cancer patients. Yet, the reasons for these associations are unclear. We show that chronic stress increased lung metastasis from disseminated cancer cells 2-4-fold in mice. Chronic stress significantly altered the lung microenvironment, with fibronectin accumulation, reduced T cell infiltration, and increased neutrophil infiltration. Depleting neutrophils abolished the stress-induced metastasis, indicating neutrophils' critical role. Chronic stress shifted the normal circadian rhythm of neutrophils and, via glucocorticoids release, caused increased neutrophil extracellular trap (NET) formation. Importantly, in mice with neutrophil-specific glucocorticoid receptor deletion, chronic stress failed to increase NET levels and metastasis. Furthermore, digesting NETs with deoxyribonuclease (DNase) I prevented chronic stress-induced metastasis. Together, our data show that glucocorticoids released during chronic stress cause the formation of NETs and establishment of a metastasis-promoting microenvironment. Therefore, NETs could be targets for preventing metastatic recurrence in cancer patients, many of whom will experience chronic stress due to their disease.

Awarded

SHORT TALK

Macrophage-fibroblast JAK/STAT dependent crosstalk promotes liver metastatic outgrowth in pancreatic cancer

Meirion Raymant

Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Ashton Street, Liverpool, UK.

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and highly metastatic disease but currently no therapeutic strategies successfully inhibit metastasis. In PDAC, the liver is the main metastatic site, where macrophages and fibroblasts are critical to successful metastatic outgrowth; from the generation of a hospitable premetastatic niche, to established outgrowth and exclusion of cytotoxic T cells (Nielsen et al., NCB. 2016; Quaranta et al., Cancer Res. 2018; Mielgo & Schmid., Perspect Med CSH, 2019). However, the role and diversity of metastasis-associated fibroblasts (MAFs) and their interaction with macrophages in PDAC remains poorly understood.

Here we applied single-cell RNA sequencing to deconvolve MAF heterogeneity and crosstalk with macrophages in murine models of liver metastatic PDAC. We identified two major MAF populations with vascular (vMAFs) and myofibroblastic (myMAF) characteristics, and two minor populations of inflammatory (iMAF), and cycling MAFs (cycMAF). Macrophage depletion reshaped the MAF landscape, altering the ratio of vMAF:myMAF, which, in turn, correlated with stromal reprogramming, including reduced collagen deposition and increased tumour vascularisation.

myMAFs enriched for active JAK/STAT signalling, which was revealed to be macrophage-dependent. Pharmacological and selective genetic inhibition of myMAFSTAT3 signalling revealed a pro-tumorigenic function for pSTAT3+myMAFs. *In vitro* modelling unveiled myMAFs immunoregulatory role in promoting immunosuppressive macrophage polarisation. Further, Inhibition of pSTAT3+myMAFs *in vivo* led to fewer immunosuppressive macrophages and increased CD8+ T cell infiltration and activation. Collectively, our findings reveal a pathogenic cross-talk of macrophages and pSTAT3+myMAFs that is sensitive to pharmacological inhibition. Our results provide further evidence for stroma-targeted therapies in patients with advanced metastatic disease.

Other Information: These studies were supported by grants from Cancer Research UK (A25607, A26978, A26979), Medical Research Council (MR/P018920/1) and North West Cancer Research Fund for M.C.S, Wellcome Trust (102521/Z/13/Z) and North West Cancer Research Funds for A.M., Cancer Research UK A17196, A2996, and A25233 for J.P.M. N.C.H. is supported by a Wellcome Trust Senior Research Fellowship in Clinical Science (ref. 219542/Z/19/Z). This abstract submission was funded by the Douglas Endowment Fund, in a travel grant awarded by the Institute of Systems Molecular and Integrative Biology, University of Liverpool.

Awarded

SHORT TALK

In vivo screening of tumorhepatocyte interactions identifies Plexin B2 as a gatekeeper of liver metastasis

Constanza Borrelli
Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland

It is estimated that only 0.02% of disseminated tumor cells are able to seed overt metastases. While this suggests the presence of environmental constraints to metastatic seeding, the landscape of host factors controlling this process remains largely unknown. Combining transposon technology and fluorescent niche labeling, we developed an *in vivo* CRISPR activation screen to systematically investigate the influence of hepatocytes on metastatic seeding in the liver. Our approach enabled the identification of Plexin B2 as a critical host-derived regulator of metastasis.

Plexin B2 upregulation in hepatocytes dramatically enhances grafting in colorectal and pancreatic cancer syngeneic models, and promotes seeding and survival of patient-derived organoids. Notably, ablation of Plexin B2 in hepatocytes prevents mesenchymal-to-epithelial transition of extravasated tumor cells and thereby suppresses liver metastasis. We dissect a mechanism by which Plexin B2 interacts with class 4 semaphorins on tumor cells, activating Rac1-mediated cytoskeleton remodeling and inducing transcription downstream of Klf4, Elf3 and Ghrl2, thereby promoting the acquisition of epithelial traits. Our findings highlight the essential role of signals from the liver parenchyma for the survival of disseminated tumor cells, prior to the establishment of a growth promoting niche. They further suggest that acquisition of epithelial traits is required for the adaptation of extravasated cells to their new tissue environment. Targeting of Plexin B2 on hepatocytes shields the liver from colonizing cells and thus presents an innovative therapeutic strategy for preventing metastasis. Finally, our screening technology, which evaluates hostderived extrinsic signals rather than tumor-intrinsic factors for their ability to promote metastatic seeding, is broadly applicable and lays a framework for the screening of environmental constraints on metastasis in other organs and cancer types.

SHORT TALK

RANK pathway inhibition impairs immunosupression in macrophages enhancing the anti-tumour response

Barranco A. [1,6], Vethencourt A. [2,3,6], Perez-Chacon G. [1,6], Trinidad Em. [3], Jimenez M. [1], Pernas S. [2,3], Dorca E. [4], Petit A. [4], Soler T. [4], Urruticoechea A. [5], Alvarez R. [1], Gomez G. [1], Piñeiro E. [1], Soria G. [1], Falo C. [2,7], Gonzalez-Suarez E. [1,3,7]

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6. These Authors Contributed Equally
7. These Authors Jointly Supervised This Work

RANK expression in breast cancer (BC) cells enhances stemness and immunosuppression, supporting the immunomodulatory role of the RANKL inhibitor denosumab. Beyond BC cells, RANK is strongly expressed by tumour-associated macrophages (TAMs), which frequently infiltrate breast adenocarcinomas, but whether and how RANK influences macrophage (MØ) phenotype and functionality is unknown. Most TAMs derive from monocytes recruited from circulation and are generally associated with poor prognosis. We hypothesize that RANK expression in TAMs may contribute to generate an immunosuppressive phenotype that support BC progression and metastasis.
To this aim, the E0771 cell line that gives rise to tumours strongly infiltrated by RANK+ TAMs, was implanted in RANKLysMΔ/Δ mice, which preferentially deletes RANK in MØs. RANKLysMΔ/Δ mice present a delayed tumour onset and a tendency to reduce lung metastasis. Gene expression profile by single cell RNAseq of RANKLysMΔ/Δ intratumour myeloid cells revealed an enrichment in genes and pathways related to a pro-inflammatory TAM phenotype, including the transcription factors Stat1/Irf1, Ifn-driven genes, antigen presentation genes, leading to a T cell-mediated anti-tumour immune response.

The clinical relevance of these findings was confirmed in BC samples from the D-BIOMARK clinical trial (NCT03691311), where a denosumab-driven decrease in immunosuppressive MØs and an increase in monocytes-related genes was observed, together with enhanced activation of innate and adaptive immune pathways. These results suggest that denosumab interferes with MØ differentiation and polarization in the tumour microenvironment.
Altogether, our preclinical and clinical results demonstrate that RANK expression in myeloid cells favours BC progression and that RANKL inhibition impairs TAMs-immunosuppressive phenotype, enhancing anti-tumour immune response.

SHORT TALK

MAF amplification licenses Estrogen Receptor α to drive breast cancer bone metastasis

Roger Gomis
Institute for Research in Biomedicine, Spain

MAF-amplification, an AP-1 transcription factor, increases the risk of breast cancer (BCa) metastasis through poorly understood mechanisms but with important clinical implications. Estrogen receptor-positive (ER+) BCa depends on estrogen for both growth and metastasis, albeit by poorly known mechanisms. Here we integrate proteomics, transcriptomics, epigenomics, chromatin accessibility and functional assays derived from human and syngeneic mouse BCa models to show that MAF directly interacts with ER , thereby promoting a unique chromatin landscape that favors metastatic spread. We identify metastasis-promoting genes that are de novo licensed following estrogen exposure in a MAF-dependent manner. Central to the epigenomic remodeling that facilitates the expression of the pro-metastatic MAF/Estrogen-driven gene expression program is the histone demethylase KDM1A. Indeed, loss of KDM1A activity prevents MAF/Estrogenmediated BCa metastasis. Collectively, we disentangle the molecular framework of MAF/Estrogen-mediated metastasis and demonstrate that genetic, epigenetic and hormonal systemic cues are integrated in BCa cells to determine their metastatic success, and thereby patient prognosis

Microenvironmental Regulation of Metastasis

Johanna Joyce
University of Lausanne
Ludwig Institute of Cancer Research
Lausanne - Switzerland

Cancers develop in complex tissue environments, which they depend upon for sustained growth, invasion and metastasis. Different tissue and tumor microenvironments are populated by diverse cell types including innate and adaptive immune cells, fibroblasts, blood and lymphatic vascular networks, and specialized organ- specific cell types, which collectively have critical functions in regulating tumorigenesis. An example of an exquisitely organ-specific microenvironment is the brain, with a number of critical tissue-resident cells playing key roles in regulating brain cancer initiation, development and metastasis.We explore how reciprocal communication between cancer cells and diverse immune and stromal cell types in the tumor microenvironment regulates each of the key stages of disease progression, and the response to therapeutic intervention. We then exploit this knowledge to devise novel and effective strategies to therapeutically target the tumor microenvironment, with a special emphasis on brain cancers. Our latest research in this area will be presented.

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Session #6
CTCS/ DTC/ CTDNA:
New technologies and implications

Chairperson: ***Paloma Bragado***

Circulating Tumour Cells and Lung Cancer Metastasis

Caroline Dive
Caroline Dive PhD, CBE., CRUK Cancer Biomarker Centre, University of Manchester

Small Cell lung Cancer (SCLC) is an aggressive neuroendocrine tumour with early metastatic spread to multiple sites including liver and brain with very poor prognosis. Apart from the recent introduction of immunotherapy which provides some durable responses in a minority of patients, the standard treatment is platinum-based chemotherapy where tumour relapse swiftly follows initial response.

Tissue biopsies are challenging, restricting progress to understand the biology of this disease. To address this issue, we developed a biobank of >65 patient-derived models (termed CDX) in immune-compromised mice from their relatively prevalent circulating tumour cells (CTCs). Metastasis to sites seen in patients occurs after subcutaneous tumour resection, with certain models displaying tropism to e.g., brain or to liver. I will discuss a few of the multiple applications of CDX models including a) the discovery of a new subtype defined by ATOH1 expression, that promotes liver metastasis (unpublished), and b) how we exploited the CDX models to develop a blood test to categorise patients into the recently identified molecular subtypes that bring potential for stratified treatment1.

In the second part of my talk, I will describe our work on CTCs in the TRACERx NSCLC program2. Here the number of pulmonary vein (PV) circulating epithelial cells (CECs) some of which have genomes consistent with NSCLC), that harvested at tumour resection with curative intent predict risk of disease recurrence. Validation of this biomarker is ongoing. I will also discuss a case study where we compared sequencing data from the primary resected NSCLC, single PV CTCs at resection and a subsequent metastasis.

References: Chemi et al., Nature Cancer, 2022, Chemi et al., Nature Medicine, 2019.

Circulating tumor cell clusters

Nicola Aceto
Institute of Molecular Health Sciences (IMHS)
ETH Zürich
Zürich – Switzerland

The metastatic spread of cancer occurs through the generation and hematogenous dissemination of circulating tumor cells (CTCs). Among these, CTC clusters have recently emerged as key players in the metastatic process. With a combination of microfluidic technologies and single cell-resolution molecular analysis applied to cancer patients and mouse models, we gained fundamental insights into the biology, vulnerabilities, and clinical implications of CTC clusters. For instance, we found that their physical features enable molecular changes that promote stemness and metastasis, allowing us to define new treatment concepts aimed at suppressing their metastatic potential. Further, we investigated CTC heterogeneity at the single cell level, revealing fundamental interactions that occur between CTCs and immune cells and that accelerate metastasis formation, as well as microenvironment signals that impact on CTC generation. More recently, we also gained important insights into CTC generation dynamics and signals that trigger intravasation. Together, our findings support a model whereby upon defined microenvironmental cues, CTCs form multicellular aggregates to expand their metastatic ability, providing a new rationale for targeting these interactions in cancer.

SHORT TALK

Circulation-on-a-Chip: Cell Survival Under Pro-Apoptotic Mechanical Cues in Metastasis

Marc Rico-Pasto

Unit of Biophysics and Bioengineering, Department of Biomedicine, School of Medicine and Health Sciences, Universitat de Barcelona, Spain

Lung adenocarcinoma (ADC) is a major contributor to brain metastasis, which is a major cause of cancer death. Conventional treatments and studies have focused on primary and secondary tumors, leaving the circulating tumor cells (CTCs) understudied. Likewise, how CTCs overcome the pro-apoptotic cues posed by the hydrodynamic conditions within the circulation (shear stress, hydrostatic pressure) in their journey to the brain remains largely unknown. To address this gap of knowledge, we designed a microfluidic based circulation-on-a-chip model that reproduces key physiological hydrodynamic features of the middle cerebral artery to investigate how disseminated cancer cells, survive within this hostile mechanical environment. Using this system, we examined the survival of different lung cancer cell lines exhibiting low (H441) or high (H460) metastatic potential Cell lines with traits of monocytes (THP-1) and T-cells (Jurkat) were used as positive controls.

Our results demonstrate that the aggressive H460 cell line survives significantly more than non-aggressive H441 cells, yet all cancer cells consistently exhibited lower survival than both THP-1 and Jurkat cells at all times examined. In all conditions, cell viability as a function of time could be modeled with a simple biophysical model based on an exponential decay and an activated process corresponding to the energetic barrier that cells need to overcome to activate death response during the hydrodynamics posed by the circulation. These results provide a proof-of-principle of a novel circulation-on-a-chip model, which provides a suitable tool to study CTCs by identifying the mechanisms underlying the enhanced survival of cancer cells with high metastatic potential, by screening drugs against their aberrant survival as well as to generate models of residual disease by enriching a population of cells with enhanced survival within the circulation.

Age-related clonal hematopoiesis drives awakening of dormant breast cancer cells in the lung

Julio Aguirre-Ghiso
Albert Einstein College of Medicine, US

Most breast cancer patients succumb to metastatic relapse years to decades following primary tumor resection and reactivation of clinically dormant disseminated cancer cells (DCCs). Improved clinical management of breast cancer has increased patient survival, although this leads to metastatic relapse in aging individuals. Inflammation is associated with aging and metastatic progression, however little is known about how this impacts DCC dormancy and relapse. Sequencing studies have revealed that age-specific somatic mutations (e.g., DNMT3A, ASXL1, TET2) in hematopoietic stem and progenitor cells (HSPCs) result in clonal expansion in a process known as clonal hematopoiesis (CH), which is associated with increased inflammatory myeloid cells and breast cancer-specific mortality. We hypothesized that CH myeloid cells could shape target organs that harbor DCCs to be pro-awakening. Data from human clinical trials revealed that in ER+ and HER2+ metastatic breast cancer patients CH mutations were associated with a shorter PFS. We proposed that progression may be fueled by enhanced reawakening of DCCs by mutant CH effector cells and thus therapy is overwhelmed. To test whether CH can accelerate dormant DCC awakening we ablated common CH genes (e.g. Dnmt3a) in HSPCs and transplanted HSPCs into lethally irradiated recipients. Following HSPC engraftment, mammospheres from early lesions of the MMTV-HER2 breast cancer model (ER+/PR+/HER2+) were orthotopically implanted into wt or CH mice. Our data revealed that mutant CH mice contained a higher burden of micro-metastatic disease and increased levels of CH myeloid cells in the lung compared to wt mice. Mechanistic data on these findings will be presented at the meeting. This study provides unprecedented insights into how the aging microenvironment may be driving the progression of metastatic breast cancer through the reactivation of dormant DCCs.

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Session #7
Neurobiology of metastasis

Chairperson: ***Manuel Valiente***

Targeting neural signalling to prevent relapse after cancer treatment

Erica Sloan

Cancer Neural Immune laboratory
Monash Institute of Pharmaceutical Sciences Monash University Victoria
Australia

Sympathetic nerves in breast tumours promote tumour growth and metastasis. To explore the impact of chemotherapy on sympathetic nerves in tumours we used mouse xenograft models, and found that the anthracycline doxorubicin increased sympathetic nerve fibre density and norepinephrine neurotransmitter levels in tumours. Doxorubicin also increased β2AR expression and response in tumour cells. Chemical ablation of sympathetic nerves using neurotoxin, or pharmacological β-blockade of neural signalling, improved the effect of doxorubicin by preventing the development of metastasis in mice. Retrospective analysis of triple negative breast cancer patients showed that incidental β-blocker use was associated with reduced risk of metastatic recurrence in women who received anthracycline-containing chemotherapy, particularly in the neoadjuvant setting. These findings indicate that the anthracycline doxorubicin increases sympathetic innervation in tumours and enhances tumour cell response to neural signalling, supporting the use of strategies that block neural signalling to improve the effects of doxorubicin chemotherapy.

Metastasis

The Neurobiology of Brain Metastasis

Frank Winkler
University Hospital Heidelberg and German Cancer Research Center
Heidelberg - Germany

Our nervous system is involved in the inititation, growth, dissemination, and therapy resistance of many cancer types throughout the body. In this talk I will present recent advantages in this emerging field of "Cancer Neuroscience", with a focus on our work on metastatic brain tumors: how cancer cells hijack neurodevelopmental pathways to build communicating tumor cell networks and how they interact with blood vessels to colonize the brain; how neurons form synapses to tumor cells that stimulate tumor growth and invasion; and how neuro-cancer disconnection strategies are developed. A special focus will lie on the question how these discoveries can be optimally translated into the clinic.

Metastasis

SHORT TALK

Gap-Junction mediated calcium oscillations drive melanoma brain metastasis progression

Nils Hebach
University Hospital Heidelberg /
German Cancer Research Institute, Germany

Gap-Junctions have long been implicated in the tumour progression and metastasis of melanoma and breast cancer. Recently, in primary brain tumours connexins have been shown to enable the formation of highly organized networks, leading to increased proliferation and resistance to therapy. One hallmark of these tumour networks are spontaneous calcium oscillations travelling through the network. We therefore set out to investigate whether such oscillations and putative networks can also be found in metastases to the brain.
Employing longitudinal intravital two-photon microscopy in awake mice and *in vitro* calcium imaging, we demonstrate for the first time that melanoma brain metastases (MBrM) exhibit spontaneous calcium oscillations. We elucidated the molecular mechanism behind these oscillations and showed that they not only depend on an interplay of calcium-induced calcium release pathways and store-operated calcium entry, but also gap-junction mediated crosstalk between tumour cells. Mathematical modelling indicated that neighbouring cells exhibit similar calcium activity profiles, suggesting that brain metastases function as a communicating network. Intercellular contacts were confirmed by dye transfer experiments. Importantly, inhibition of gap junctions significantly reduced calcium oscillations *in vitro*, as well as tumor proliferation both *in vitro* and *in vivo*. Our current focus is on uncovering the downstream pathways activated by these calcium oscillations to identify actionable targets and enhance our understanding of melanoma brain metastases biology.

Metastasis

Humsa Venkatesh
Brigham and Women's Hospital
Harvard Medical School
Boston, US

Neural activity is increasingly recognized as a critical regulator of cancer growth. In the brain, neuronal activity robustly influences glioma growth both through paracrine mechanisms and through electrochemical integration of malignant cells into neural circuitry via neuron-to-glioma synapses, while perisynaptic neurotransmitter signaling drives breast cancer brain metastasis growth. Outside of the CNS, innervation of tumors such as prostate, breast, pancreatic and gastrointestinal cancers by peripheral nerves similarly regulates cancer progression. However, the extent to which the nervous system regulates lung cancer progression, in the lung or when metastatic to brain, is largely unexplored. Small cell lung cancer (SCLC) is a lethal high-grade tumor originating from pulmonary neuroendocrine cells that exhibits a strong propensity to metastasize to the brain. Here we demonstrate that metastatic SCLC cells in the brain similarly co-opt mechanisms of neural plasticity to stimulate growth and progression. Optogenetic stimulation of cortical neurons drives proliferation and invasion of SCLC brain metastases. Neuronal activity induces electrical currents and consequent calcium transients in SCLC cells. Direct membrane depolarization is sufficient to promote SCLC growth in the brain. In the lung, vagus nerve transection markedly inhibits primary lung tumor formation, progression and metastasis, highlighting a critical role for innervation in overall SCLC initiation and progression. Taken together, these studies illustrate that neuronal activity plays a crucial role in dictating small cell lung cancer pathogenesis in both primary and metastatic sites

Neural basis for blunted circadian glucocorticoid rhythms in breast cancer

Jeremy Borniger
Cold Spring Harbor Laboratory
New York, US

Breast cancer is the most common malignancy in women, comprising approximately 30% of all new female cancers each year. Although we have made substantial progress in early detection and therapy, nearly all patients that develop metastatic disease will succumb to it. Therefore, there is critical need for innovative approaches that aim to reduce relapse, prevent metastasis, and bolster existing therapies. We sought to investigate a well-described predictor of breast cancer mortality and quality of life, i.e., progressive blunting of circadian rhythms in circulating glucocorticoids (GCs) during cancer progression. Specifically, patients with 'flat' GC slopes (indicating little difference between morning and evening GC concentrations) succumb significantly earlier to cancer than patients with robust circadian GC rhythms. Despite knowing of this phenomenon for several decades, we still do not know why or how cancer influences GC rhythms. To address this gap in knowledge, we monitored circadian GC rhythms throughout the course of tumor progression in multiple mouse models of breast cancer (syngeneic, orthotopic E0771 cells; autochthonous MMTV-PyMT model, and tail vein MMTV-PyMT-lung met model). Tumor bearing mice exhibited a 40-50% blunting of the natural GC rhythm, a phenotype that emerged even before tumors were palpable. Mice displayed normal adrenal and pituitary gland function, but exhibited aberrant activity of hypothalamic neurons that control GC production (i.e., PVNCRH neurons). Enrichment and RNA-sequencing of these neurons revealed large changes in the expression of transcripts related to neuronal excitability and synapse formation (e.g., Malat1), which was consistent with changes in excitatory synapse density on these neurons. Additionally, artificial enforcement of robust GC rhythms via chemogenetic stimulation of PVNCRH neurons reduced primary tumor growth, an effect that depended on circadian phase. Ongoing work is focused on understanding how tumor progression changes the electrophysiological properties of these neurons, and determining how neuromodulation of these cells attenuates tumor progression.

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Organisers & Speakers' Biographies

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Julio Aguirre-Ghiso

Cancer Dormancy and Tumor Microenvironment Institute, Gruss-Lipper Biophotonics Center, Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine, Montefiore Einstein Cancer Center, Albert Einstein College of Medicine
New York, US

Julio A. Aguirre-Ghiso, PhD is the Rose Falkenstein Chair in Cancer Research, Professor of Cell Biology and Founding Director of the Cancer Dormancy & Tumor Microenvironment Institute at the Montefiore Einstein Comprehensive Cancer Center (MECCC) in New York City, where he also co-directs the Gruss-Lipper Biophotonics Center and the Tumor Microenvironment and Metastasis Program at the MECCC. Dr. Aguirre-Ghiso received his PhD from the University of Buenos Aires, Argentina in 1997 and was a Charles H. Revson post-doctoral Fellow at Icahn School of Medicine at Mount Sinai (ISMMS) until 2003. He became an Assistant Professor at SUNY-Albany the same year and since 2008 he was at ISMMS where he grew through the ranks becoming Professor in 2014 and Endowed Mount Sinai Chair of Cancer Biology in 2020. His research team led a paradigm shift, revealing novel cancer biology that diverges from the notion that cancer is perpetually proliferating. His team discovered that reciprocal crosstalk between disseminated tumor cells and the microenvironment regulates the inter-conversion between dormancy and metastasis initiation. His lab also provided a mechanistic understanding of the process of early dissemination and revealed how it contributes to dormancy and metastatic progression and how stress adaptive pathways allow cancer cells to persist while quiescent. His work has been published in renowned journals and has been highly cited. He has served and actively advises different scientific and industry entities and scientific journals such as the NIH study sections where he was elected as a permanent member, the Pershing Square Sohn Research Alliance, Cancer Cell , the French National Cancer Institute and the Metastasis Research Society among others. In 2019 he founded a new company, HiberCell and has enabled clinical approaches that seek to kill stress signaling-dependent proliferative or dormant cancer cells and/or induce cancer cell dormancy. He served as President Elect and then President of the Metastasis Research Society from 2018-2022 and has served at several leadership levels at AACR among other organizations.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Caroline Dive

CRUK Manchester Institute
Manchester, UK

Professor Caroline Dive CBE, PhD, FBPhS, FMedSci
After completing her PhD studies in Cambridge, Professor Caroline Dive moved to Aston University's School of Pharmaceutical Sciences in Birmingham where she established her own group studying mechanisms of drug induced tumour cell death, before moving to The University of Manchester to continue this research. Caroline was awarded a Lister Institute of Preventative Medicine Research Fellowship before joining the CRUK Manchester Institute in 2003. Currently, she is Interim Director of the Institute and Director of the CRUK Cancer Biomarker Centre, with research spanning tumour biology, preclinical pharmacology, biomarker discovery, biomarker assay validation and clinical qualification to regulatory standards, bioinformatics, biostatistics and most recently, digital clinical trials.

Eva Gonzalez

Transformation and Metastasis Group
Molecular Oncology Programme
Spanish National Cancer Research Centre - CNIO
Madrid, Spain

Dra. Eva Gonzalez is PhD in Molecular Biology, is a Senior scientist and group leader of the Transformation and Metastasis laboratory, at the Spanish National Cancer Research Center (CNIO) in Madrid, Spain.

Her academic merits and research have been recognized with an ERC-Consolidator grant, ERC-Proof of Concept, a Susan Komen career catalyst grant, and several awards. She is member of experts committee in several institutions as the Agencia Estatal de Investigación, European network of breast development and cancer (ENBDC), among others, and has supervised several PhD students and postdocs.

Research in her laboratory aims to identify novel targets for breast cancer treatment and understand mechanisms of resistance to current drugs. She has more than 50 publications, half of them in D1 and half as corresponding author. To highlight a topic of her extensive research career, one of her main contributions of research has focused on the role of RANK in mammary epithelial homeostasis, breast cancer and tumor immunology, with studies from basic to clinical research, including the coordination of a clinical trial in breast cancer patients.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Hector Peinado

Microenvironment & Metastasis Group
Molecular Oncology Programme
Spanish National Cancer Research Centre – CNIO
Madrid, Spain

Dr. Peinado is the Group Leader of the Laboratory of Microenvironment and Metastasis at CNIO. His laboratory is investigating the role of tumor-secreted extracellular vesicles in pre-metastatic niche formation, their potential applications in liquid biopsies and the influence of the microenvironment in metastatic dissemination. His contributions to the field have been recognized with the 1st ASEICA Young Investigator, Pfizer, and "Doctores Diz Pintado" awards. He has been honored as a FERO, Marie Curie-WHRI-Academy and BBVA Leonardo Fellow. Dr. Peinado has published 106 works since 2003 and he was listed in 2022 among the top 2% of most cited scientists worldwide, according to the Stanford University ranking based on Scopus.

Manuel Valiente

Brain Metastasis Group
Molecular Oncology Programme
Spanish National Cancer Research Centre – CNIO
Madrid, Spain

Dr. Manuel Valiente investigates the biology of brain metastasis in order to challenge this unmet clinical need with a main focus on the microenvironment. Dr. Valiente has co-founded the Spanish National Network of Brain Metastasis (RENACER), which is allowing his team to translate laboratory findings to clinical studies faster. His contributions to brain metastasis research have been recognized with the Banco Sabadell Award, ASPIRE Award, ERC CoG, EMBO YIP, CLIP Award, Beug Foundation's Prize, BMS-MRA Young Investigator Award among others. He is the Chair of the Scientific Committee of the EANO, Board Member of the MRS and ESMO faculty member.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Nicola Aceto

Institute of Molecular Health Sciences (IMHS)
ETH Zürich
Zürich – Switzerland

Nicola Aceto is Professor of Molecular Oncology at the ETH Zurich. Recent discoveries of the Aceto lab include insights into the biology circulating tumor cells and their clusters, some of which already translated in clinical trials. He obtained a PhD summa cum laude from the Friedrich Miescher Institute in Basel, and worked as a postdoctoral fellow at Harvard Medical School and MGH Cancer Center in Boston. He is recipient of the Pezcoller Foundation-EACR Translational Cancer Researcher Award (2023), the Swiss Science Prize Latsis for groundbreaking cancer research (2021) and the Friedrich Miescher Award for Outstanding Achievements in Biochemistry (2020).

ORGANISERS & SPEAKERS' BIOGRAPHIES

Eduard Batlle

ICREA & Cancer Science Program, Institute for Research in Biomedicine (IRB Barcelona).
The Barcelona Institute of Science and Technology (BIST)
Barcelona, Spain

Eduard Batlle is an ICREA professor and chair of the Cancer Science program at the Institute for Research in Biomedicine (IRB) in Barcelona, Spain. His laboratory investigates the mechanisms that drive colorectal cancer initiation and progression. Amongst other findings, his research originally identified a role for EphB/ephrin signaling in intestinal cell positioning and the connection between the intestinal stem cell program and colorectal cancer. More recently, Batlle revealed a key role for TGF-beta signaling in stromal cells during metastatic colonization. His laboratory is currently focused on understanding disease relapse and developing new prognostic and therapeutic tools for advanced colorectal cancer. His track record has been recognized through several awards/honors such as the Debiopharm Life Science Award (2006), Josef Steiner Cancer Research Award (2013), three consecutive ERC Grants (2007, 2013, 2019), the Pezcoller foundation-EACR award (2014), the Lilly Foundation Award for Preclinical research (2016) or the King Jaime-I award (2021).

Metastasis

Adrienne Boire

Memorial Sloan Kettering Cancer Center

Adrienne Boire, M.D., Ph.D. is the Geoffrey Beene Junior Faculty Chair at Memorial Sloan Kettering Cancer Center in New York. As a neuro-oncologist, she cares for patients with metastasis to the central nervous system (CNS), with particular focus on leptomeningeal metastasis. As a scientist, she runs a laboratory-based research program focused on leptomeningeal metastasis. Her team employs multi-omic analysis of human samples to identify cancer cell adaptations to the challenging microenvironment of the leptomeninges. Leveraging mouse models, the team uncovers the mechanistic implications of these discoveries to establish novel therapies for leptomengingeal metastasis

ORGANISERS & SPEAKERS' BIOGRAPHIES

Jeremy C. Borniger

Cold Spring Harbor Laboratory
New York, US

Dr. Jeremy Borniger is assistant professor of neuroscience and a member of the cancer center at Cold Spring Harbor Laboratory in New York. He received his PhD in neuroscience from The Ohio State University and completed a BRAIN Initiative postdoctoral fellowship at Stanford University in systems neuroscience before starting his laboratory at CSHL in early 2020. His laboratory focuses on understanding interactions between the nervous system and cancer at multiple spatiotemporal scales, with a specific emphasis on understanding how the brain senses, integrates, and responds to cancer-induced changes in systemic physiology.

Shang Cai

Shang Cai Ph.D. Assistant Professor of Westlake University
Westlake University, China

Dr. Shang Cai is currently the assistant professor in Westlake University. He received his bachelor degree of biological science in Peking University in 2003. He then went abroad to the Biochemistry Department of Indiana University for his PhD studies for mechanism of cell division. He pursued his postdoc research in the Institute of Stem Cell and Regenerative Medicine, Stanford University, working on the mechanism of self-renewal and fate specification of mammary stem cell and breast cancer stem cell. He joined Westlake University as assistant professor in 2017. Dr. Shang Cai's research focuses on the microenvironment regulation of stem cell fate and cancer metastasis with particular interest in the role of tissue resident microbiota.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Carlos Caldas

Cancer Research UK Cambridge Institute
University of Cambridge
Cambridge
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Carlos Caldas MD FACP FRCP FRCPath FMedSci
Carlos Caldas is Professor of Cancer Medicine at the University of Cambridge, and Head of the Breast Cancer Functional Genomics Laboratory at the Cancer Research UK Cambridge Institute. He is an Honorary Consultant Medical Oncologist and was the Founding Director of the Breast Cancer Programme at the Cambridge Cancer Centre. He is Fellow of the Academy of the Medical Sciences, Fellow of the European Academy of Cancer Sciences, and EMBO Member. He received the 2016 ESMO Hamilton Fairley Award, the 2021 European Society of Human Genetics Award, the 2021 Susan G. Komen Brinker Award for Scientific Distinction in Basic Science and the 51st Leopold Griffuel Award in 2023. He has published over 450 manuscripts, including in Nature, New England Journal of Medicine, Nature Genetics, Nature Medicine, Nature Cancer, Cell, Cancer Cell, Cell Reports, Science Translational Medicine, and Nature Communications. His research focus is the functional genomics of breast cancer and its biological and clinical implications. His laboratory redefined the molecular taxonomy of breast cancer, revealing novel subtypes and their respective drivers and multi-omic landscapes, robustly validated this new molecular taxonomy, and showed that it determines the clinical trajectories of patients. He led the studies that established ctDNA as a monitoring biomarker in breast cancer and as a liquid biopsy to unravel therapy resistance. His laboratory pioneered and developed the use of patient-derived tumour explants as models of breast cancer, in particular as a platform to characterize and perturb tumour ecosystems. In a recent landmark paper published in Nature, using multi-omics and machine learning, his group showed the biology of breast tumour ecosystems determines response to therapy. He has been distinguished by Web of Science as a Highly Cited Researcher in 2018, 2019, 2020, 2021 and 2022.

Mikala Egeblad

Cold Spring Harbor Laboratory

Mikala Egeblad, Ph.D. is a Bloomberg Distinguished Professor at Johns Hopkins University (JHU), co-leader of the Sydney Kimmel Comprehensive Cancer Center Program on Cancer Invasion and Metastasis, and chair-person of the American Association for Cancer Research Tumor Microenvironment Working Group. Her research group studies how the tumor microenvironment contributes to therapy resistance and metastasis, focusing on the roles of neutrophils and macrophages. She was previously at Cold Spring Harbor Laboratory (2009-2023). She has received the Department of Defense Breast Cancer Research Program Era of Hope Scholar Award; The Pershing Square Sohn Prize for Young Investigators in Cancer Research; and the Suffrage Science Award.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Arkaitz Carracedo

CICbioGUNE, Vizcaya, Spain

Dr. Arkaitz Carracedo received his PhD in Biochemistry and Molecular Biology from the Complutense University of Madrid in 2006. After 4 years of postdoctoral work at Memorial Sloan-Kettering Cancer Center Hospital and Beth Israel Deaconess Medical Center, Harvard Medical School (USA), he established his research line at CIC bioGUNE at the end of 2010 with the primary objective of studying the unique biological characteristics of cancer cells in vitro and in vivo, with emphasis on cell metabolism alterations. Since 2011 he is Ikerbasque Research Professor and, since 2012, Associate Professor at the University of the Basque Country. He has influenced the field of cancer metabolism with 125 scientific publications and reviews and the organization of internationally recognized congresses. This, in turn, has granted him a variety of awards and recognitions, as well as the funding of his research by the most important agents in the national and international scene, from foundations such as the AECC to the European Research Council with 3 ERC projects.

Cyrus Ghajar

Translational Research Program
Fred Hutchinson Cancer Center
Washington, US

Cyrus Ghajar, PhD is a Professor within the Translational Research Program at the Fred Hutchinson Cancer Center, where he holds the Peter S. Lefkarites Memorial Endowed Chair.

At the Hutch, he directs that the Laboratory for the Study of Metastatic Microenvironments (URL: http://research.fhcrc.org/ghajar/en.html), which aims to understand how tissue microenvironments regulate the phenotype of disseminated tumor cells (DTCs). Specifically, his laboratory is working to understand how niches throughout the body influence the survival, growth, therapeutic resistance and immune surveillance of DTCs, and how local and systemic changes impact their colonization potential. His ultimate goal is to translate findings from his laboratory to prevent metastasis in patients.

More recently, Professor Ghajar was appointed as the founding director of the Center for Metastasis Research eXcellence (MET-X) at the Hutch. The mission of this new Center is the creation of financial and intellectual infrastructure necessary for the conduct of innovative, interdisciplinary research aimed at curing Stage IV/metastatic disease.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Claudia Gravekamp, PhD

Microbiology and Immunology
Albert Einstein College of Medicine
New York - US

Claudia Gravekamp, PhD, is an Associate Professor in the Department of Microbiology and Immunology of Albert Einstein College of Medicine in New York, and member of the Albert Einstein Cancer Center. She received her PhD in 1988 in the field of Tumor Immunology at the Erasmus University in Rotterdam, The Netherlands. From 1987-1993, she served as head of the Laboratory for Leptospirosis at the Royal Tropical Institute in Amsterdam, The Netherlands. From 1993-1998, she was a Research Fellow/Instructor in Medicine at the Channing Laboratory of Harvard Medical School, Boston, MA, where she gained expertise in the development of bacterial vaccines. From 1998-2006, she was an Associate Member of the Cancer Therapy and Research Center, in San Antonio, TX, where she began to develop a program aimed at cancer vaccines. From 2006-2008, she was a Scientist at the California Pacific Medical Center Research Institute in San Francisco, CA, continuing to develop novel immunotherapeutic approaches to cancer utilizing an attenuated bacterium Listeria monocytogenes as selective delivery platform for anti-cancer agents. She has been funded by grants from NIH (RO1/R21/RO3), the pancreatic cancer action network (PCAN), and private industry, published over 80 scientific articles, is reviewer for various scientific journals, and served as reviewer on several NIH/DOD study sections. She has three patents granted on Listeria-recall antigens and Radioactive Listeria in the US and abroad.

Johanna Joyce

University of Lausanne
Ludwig Institute of Cancer Research
Lausanne - Switzerland

Dr. Johanna Joyce is a Professor at the University of Lausanne, Switzerland, and Full Member of the international Ludwig Institute for Cancer Research. Her contributions to cancer research have been recognized through the EACR-Pezcoller Award for Women in Cancer Research Award, Robert Bing Prize, Cloetta Prize, American Cancer Society Scholar Award, Rita Allen Foundation Award, V Foundation Award, Sidney Kimmel Foundation Award, among others. She is an EMBO Member, EACS Fellow, and previous Chair of the AACR Tumor Microenvironment Working Group and Member of the AACR Women in Cancer Research Council. Johanna is a committed mentor and advisor, and a widely-recognized advocate - including for women in cancer research.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Jean-Christophe Marine

Professor at KU Leuven
Director of the VIB center for Cancer Biology
Leuven - Belgium

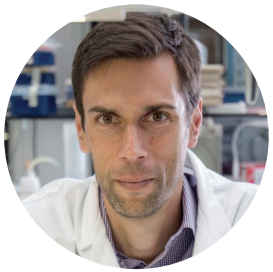
Jean-Christophe (Chris) Marine is Professor at KU Leuven (Belgium), senior VIB group leader and Director of the VIB center for Cancer Biology. He received numerous national and international awards for his work on cytokine signaling and cancer biology. He, for instance, received the outstanding research award from SMR in 2019 and was elected EMBO member in 2020. His interests focus on the mechanisms by which cancer-specific non-mutational (i.e. epigenetic and (post-) transcriptional) events shape tumour evolution. Using innovative genetic tools and leveraging cutting-edge technologies such as single-cell multiomics, spatial transcriptomics and proteomics, the Marine lab has made several key contributions to our understating of melanoma biology and, in particular, the mechanisms underlying melanoma initiation, growth, metastatic dissemination, emergence of inter-and intra-tumor heterogeneity, plasticity and resistance to both targeted and immune checkpoint therapy.

Maria Rescigno

Vice Rector and delegate for research
Humanitas University, Pieve Emanuele
Milan – Italy

Maria Rescigno (H-index 68) is full professor, vice-rector and delegate to research at Humanitas University and group leader at Humanitas Research hospital, Milan. She graduated in Biology in the University of Milan and received her PhD in Pharmacology and toxicology. She worked at the University of Cambridge, UK, as a visiting scholar. From 2001 to 2017 she has been the director of the Dendritic cell biology and immunotherapy Unit at the Department of Experimental Oncology at the European Institute of oncology. She was the first to show that dendritic cells actively participate to bacterial uptake in the gut and the existence of a gut vascular barrier that resembles the blood brain barrier. She authored more than 160 publications in high impact journals. She was nominated EMBO young investigator in 2007. In 2008-2013 she was visiting professor at the University of Oslo. In 2011 Maria Rescigno was elected EMBO member. She has been the recipient of three ERC grants (starting, proof-of-concept and consolidator). From 2019 she is member of the EMBO council. In 2016 Maria Rescigno founded Postbiotica s.r.l. a spin-off of the University of Milan that exploits microbiota-derived metabolites as new pharmaceutical agents.
She received several awards, including an honourable mention from Belgian Embassy (2022) and the prestigious Premio Roma per lo sviluppo del Paese (2022).

ORGANISERS & SPEAKERS' BIOGRAPHIES



Erik Sahai

The Francis Crick Institute
London - UK

Erik Sahai PhD is the head of the Tumour Cell Biology laboratory at the Francis Crick Institute in London. 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). Following this training, Erik set up his own group at the Cancer Research UK London Research Institute in 2004 and then transferred to the Francis Crick Institute in 2015. His research is focused on the spread of cancer through the body and responses to cancer therapy. In particular, his group is interested in stromal fibroblasts and their interplay with both tumour and immune cells. 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's contributions have been recognised by the award of the Hooke Medal, and election to EMBO, the European Academy of Cancer Sciences, and the Academy of Medical Sciences. He is president elect of the Metastasis Research Society and serves on the editorial board of several journals, including Journal of Cell Biology, Developmental Cell, and Trends in Cell Biology.

Research Interests: tumour microenvironment, optical imaging, cell-cell communication, metastasis, therapy failure and cancer evolution
https://www.crick.ac.uk/research/labs/erik-sahai
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Erica Sloan

Cancer Neural Immune laboratory
Monash Institute of Pharmaceutical Sciences Monash University Victoria
Australia

Professor Erica Sloan leads the Cancer Neural Immune laboratory at Monash Institute of Pharmaceutical Sciences at Monash University in Australia. Her research program investigates how the sympathetic nervous system impacts cancer progression. Using advanced imaging technologies in preclinical models of cancer, she defined how chronic stress acts through neural signalling to control the behaviour of both cancer cells and immune cells, to affect cancer metastasis. Her team recently completed a Phase II randomised controlled trial that defined the effect of blocking neural signalling in breast cancer patients. Her team are currently applying this knowledge to improve standard cancer treatments.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Humsa Venkatesh

Brigham and Women's Hospital
Harvard Medical School
Boston, US

Humsa Venkatesh is an Assistant Professor in Neurology at the Brigham and Women's Hospital and Harvard Medical School. Her research studies the electrical components of tumor pathophysiology and highlights the extent to which neural activity controls and facilitates disease progression. The understanding of these co-opting mechanisms has led to novel strategies to broadly treat cancers, by disabling their ability to electrically integrate into neural circuitry. Her pioneering research in this emerging field of cancer neuroscience aims to harness the systems level microenvironmental dependencies of tumor growth to develop innovative therapeutic treatments.

Alana Welm

The University of Utah
Utah, US

Dr. Welm completed her PhD at Baylor College of Medicine and postdoctoral training in J. Michael Bishop's laboratory at the University of California, San Francisco. Dr. Welm started her laboratory at the University of Utah's Huntsman Cancer Institute (HCI) in 2007, which is focused on metastatic breast cancer. She now holds the Ralph E. and Willia T. Main Presidential Endowed Chair in Cancer Research and is Senior Director of Basic Science at HCI. She is the PI on multiple grants from the National Cancer Institute and DOD, and has received DOD Era of Hope and Susan G. Komen Scholar awards.

ORGANISERS & SPEAKERS' BIOGRAPHIES

Frank Winkler

University Hospital Heidelberg and German Cancer Research Center
Heidelberg - Germany

Professor Frank Winkler is a managing senior physician in the Department of Neurology at the University of Heidelberg and group leader at the German Cancer Research Center. He studied medicine in Hamburg, Freiburg and London, specialized in Neurology at the LMU Munich, spent a 2 year postdoc at Harvard, and was appointed to Heidelberg in 2010. Dr Winklers' work has been published in Nature, Cell, Nature Medicine, Cancer Cell. In 2022 he received the German Cancer Award.

His work focusses on the interaction of the nervous system with cancer, pioneering the field of Cancer Neuroscience, and launching investigator-initiated trial concepts.

Metastasis

Xiang H.-F. Zhang

Professor of Molecular and Cellular Biology
The William T. Butler, M.D.
Endowed Chair for Distinguished Faculty Interim Director,
Lester and Sue Smith Breast Center McNair Scholar
Baylor College of Medicine - Houston - US

Dr. Xiang Zhang obtained his Ph.D. from Columbia University in 2006, and did his postdoctoral training at MSKCC In 2011, he was recruited to Baylor College of Medicine as a McNair Scholar, and is now the William T. Butler Endowed Professor and Director of Breast Center. His lab focuses on metastatic breast cancer in two areas: 1) bone metastasis and 2) tumor immunology and immunotherapies. Dr. Zhang has published over 100 papers in these fields including first- or senior authored papers on *Cell, Nature, Cancer Cell, Cell Stem Cell, Nature Cancer, Nature Cell Biology, and Developmental Cell.*

cnio - CaixaResearch FRONTIERS MEETINGS

Madrid 6th – 8th November 2023

Metastasis

Poster Session

cnio stop cancer

01

Investigating BACH1 as a potential anti-metastatic therapeutic target in cancer

Kevin Ali[1, 2], Samantha Alvarez[1, 2], Angana Patel[1, 2], Jozefina Dzanan[1, 2], Ilayda Altinönder[1, 2], Laureano De la Vega[3], Clotilde Wiel[1, 2], Volkan Sayin[1, 2].

1. Sahlgrenska Cancer Center, Department of Surgery, Institute of Clinical Sciences, University of Gothenburg, Gothenburg, Sweden.
2. Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden.
3. Jacqui Wood Cancer Centre, Division of Cellular Medicine, School of Medicine, University of Dundee, UK.

Lung cancer is the deadliest cancer type, primarily because metastasis has occurred. Currently, there are no specific treatments to block metastasis. Studies indicate that cancer cells metastasize by rewiring metabolic pathways and adapting to oxidative stress, by activating the antioxidant transcription factor NRF2. 20-30% of patients with non-small cell lung cancer (NSCLC) carry gain-of-function mutations in NRF2 or loss-of-function mutations in KEAP1, the negative regulator of NRF2. Activated NRF2 is known to promote KRAS-driven NSCLC metastasis.
BACH1 is a pro-metastatic transcription factor and heme sensor that is inhibited by high levels of heme. NRF2 activation reduces heme levels, leading to the stabilization of BACH1, which activates a pro-metastatic program. Knocking-out BACH1 in cancer cell lines impaired migratory and invasive capabilities. Consequently, targeting BACH1 can potentially prevent metastasis.
The number of existing BACH1 inhibitors is limited. Recently, we identified cannabidiol (CBD) and Bardoxolone Methyl Ester (CDDO-Me) as BACH1 inhibitors that specifically decrease nuclear BACH1 levels and decrease cancer cell invasion/migration *in vitro*. Concurrently, we found that KEAP1 mutant cancers respond better to BACH1 inhibitors due to their increased dependence on stabilized BACH1. Thus, inhibiting BACH1 in KEAP1 mutant cancers more profoundly decreases cell migratory capabilities and metastatic potential. Further testing was conducted *in vivo* using a mouse model of metastasis by injecting KEAP1 mutated murine cells into mice. The mice treated with BACH1 inhibitors showed reduction in metastatic burden, providing further support for BACH1 as a potential anti-metastatic target. Future work includes assessing and validating other novel BACH1 inhibitors in various metastasis models and cancer types to determine whether BACH1 is a viable therapeutic target for metastasis.

POSTER SESSION

02

A multidimensional model analysis to dissect the impact of brain metastasis on neuronal communication

Al-Masmudi-Martín M.[1], Sanchez-Aguilera A.[2], Navas-Olive A.[2], Baena P.[1], Hernández-Oliver C.[1], Priego N.[1], Cordón-Barris L.[1], Alvaro-Espinosa L.[1], García S.[1], Martínez S.[1], Lafarga M.[3], RENACER[4], Lin MZ.[5], Tezanos P.[2], Muela P.[2], Cintado E.[2], Trejo JL.[2], Al-Shahrour F.[1], Menendez de la Prida L.[2], Valiente M.[1].

1. Spanish National Cancer Research Center (CNIO), Madrid, Spain,
2. Instituto Cajal. CSIC, Madrid, Spain,
3. University of Cantabria- IDIVAL, Santander, Spain,
4. Red Nacional de Metástasis Cerebral,
5. Stanford University, Stanford, CA.

A high percentage of patients with brain metastases frequently develop neurocognitive symptoms, however understanding how brain metastasis co-opt the function of neuronal circuits beyond the mass effect remains unknown. We report a comprehensive multidimensional modelling of brain functional analysis in the context of brain metastasis. By testing different pre-clinical models of brain metastasis from various primary sources and oncogenic profiles, we dissociated the heterogeneous impact on brain function that we detected from the homogeneous inter-model tumor size. In contrast, we report a potential underlying molecular program responsible for impairing neuronal crosstalk in a model-specific manner. Additionally, measurement of various brain activity readouts matched with machine learning strategies confirmed a novel biomarker to predict the presence of metastases and the subtype. Finally, as in patients, the inter-model heterogeneity regarding brain activity impact correlated with failures in specific behavioral tests. We envision that our findings not only increase our knowledge on the molecular basis of neurocognitive impairment associated with brain metastases but they are also the first step towards a new therapeutic strategies to prevent or stop the decline in quality of life associated with these symptoms. In addition, our computational findings exploiting electrophysiological profiles suggest the possibility to exploit them as novel biomarkers.

03

Forces driving benign-to-invasive transition in squamous cell carcinoma

Jorge Almagro, Elaine Fuchs

1. Robin Chemers Neustein Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, New York, NY, USA
2. Howard Hughes Medical Institute, New York, NY, USA

Cells, molecules and mechanical forces that constitute the tumour microenvironment play a critical role in tumour progression. In cutaneous carcinomas the difference between a benign and an invasive lesion is defined by whether the cancer cells cross the basement membrane (BM). Multiple factors contribute to the breaching of the BM including protease-secreting cells, de novo BM formation and forces exerted by the tumour and the TME. Using advanced imaging techniques including tissue clearing and 3D immunofluorescence, electron microscopy and atomic force microscopy we interrogate which stromal cells of the TME regulate the integrity of the BM throughout squamous cell carcinoma (SCC) development.

POSTER SESSION

04

ADAMTS5 and Integrin crosstalk in controlling ovarian cancer migration and invasion

Rachele Bacchetti, Jamie Adams, Shengnan Yuan and Elena Rainero

School of Biosciences, The University of Sheffield

Ovarian cancer is the most common cause of female gynaecologica cancer death. 80% of patients will have developed metastasis at diagnosis. A better understanding of the early metastatic events is necessary. Ovarian cancer metastasis relies on cancer cells migration and interaction with the extracellular matrix (ECM). Matrixdegrading enzymes like ADAMTS are fundamental to degrade the ECM and improve migration, leading to the formation of a tumour-promoting environment. Thanks to cell surface receptors like integrins, cancer cells regulate cell-matrix adhesion. Rab25 have been shown to control cancer cell migration in 3D environments, increasing integrin recycling. A microarray analysis showed that ADAMTS5 is upregulated by Rab25 in 3D but not 2D environments. We have shown that treating ovarian cancer cells (A2780) expressing Rab25 with ADAMTS5 inhibitor reduces directional cell migration. We therefore hypothesised that overexpression of Rab25 leads to higher recycling of α 5 β 1 integrin, which in turn contributes to the upregulation of ADAMTS5. Here we showed that fibronectin promotes ADAMTS5 expression, and we are currently investigating the role of the fibronectin receptor α 5 β 1 integrin in controlling this. We are also developing an *in vitro* and an *in vivo* model to characterise the migratory behaviour and elucidate possible inhibitory molecules/drugs targeting ADAMTS5 activity/regulation.

05

Development of novel scFv based molecules to interfere the metastatic process

Rocío Bayona-Ramón y Cajal[1], Elena Muro-Blanc[2], Stefan Hümmer[2], Alberto J. Schuhmacher[1,3], Santiago Ramón y Cajal[2,4].

1. Aragón Health Research Institute (IIS Aragón), Molecular Oncology Group, Zaragoza, Spain.
2. Translational Molecular Pathology, Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.
3. Fundación Agencia Aragonesa Para la Investigación y el Desarrollo (ARAID), 50018 Zaragoza, Spain
4. Spanish Biomedical Research Network Centre in Oncology (CIBERONC),Madrid, Spain.

In recent years, there have been significant improvements in the management of breast cancer. However, metastatic breast cancer continues to present a discouraging prognosis, with 5-year survival rates plummeting below 20%. In the pursuit of understanding resistance to tumor stress, extensive screening has revealed numerous molecular components at play. Particularly, the uptake of extracellular vesicles (EVs) has emerged as a critical element in intracellular communication, integral to the progression of breast cancer metastasis. Identifying the molecular agents involved in the uptake of EVs by breast cancer cells allows for *in vitro* and *in vivo* screening to identify selective inhibitors that prevent this process and, therefore, may delay or prevent the progression of breast cancer metastasis. We have optimized a high troughput system that allows the real time and simultaneous monitoring of drugs that are likely to inhibit the EV uptake. Among the plethora of molecules that disrupt the metastasis-promoting process, we have successfully identified and produced a specific single-chain variable fragment (scFv). Preliminary experiments with this scFv have demonstrated promising results, suggesting its potential in obstructing EV internment and consequently impeding metastatic spread. This inhibitor showcases encouraging potential in its ability to interfere with the metastatic process.

POSTER SESSION

06

Interrogation of endothelial and mural cells in brain metastasis by multiomic analysis reveals key immune-regulatory mechanisms

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Brain metastasis (BrM) represents the most common brain malignancy in adults, predominantly originating from lung cancer, breast cancer, and melanoma. Recent studies have revealed the importance of the brain tumor microenvironment (TME), particularly diverse immune cells, in regulating cancer progression in both primary and metastatic brain malignancies. The blood-brain barrier (BBB) is another critical TME component formed by endothelial cells, mural cells, astrocytic end-feet, and closely-associated microglia. Metastasizing cancer cells can use different strategies to traverse the BBB and may subsequently exploit the vasculature for their own benefit, to create the blood-tumor barrier. To investigate the mechanisms underlying vascularization in brain metastasis we performed a comprehensive multiomic analysis of the key components of the tumor vasculature. We integrated single-cell and/or bulk RNA sequencing of sorted endothelial and mural cells isolated from human BrM of different origins, multiple mouse BrM models, and non-tumor brain samples; immunofluorescence imaging analysis of the TME spatial architecture; and functional studies using mouse BrM models to target vascular regulators of tumor immunity. Moreover, from our preclinical trials, we have revealed promising new therapeutic strategies for these aggressive tumors. Collectively, our results have revealed important new insights into the biology underlying vascularization in metastatic brain tumors, with a particular emphasis on the critical role of vascular cells as immune regulators.

07

Breast adipose tissue-derived extracellular vesicles from women with obesity stimulate mitochondrial-induced dysregulated tumor cell metabolism

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Breast adipose tissue is an important contributor to the obesity-breast cancer link. Dysregulated cell metabolism is now an accepted hallmark of cancer. Extracellular vesicles (EVs) are nanosized particles containing selective cargo, such as miRNAs, that act locally or circulate to distant sites to modulate target cell functions. Here, we found that long-term education of breast cancer cells (MCF7, T47D) with EVs from breast adipose tissue of women who are overweight or obese (O-EVs) leads to sustained increased proliferative potential. RNA-Seq of O-EV-educated cells demonstrates increased expression of genes, such as ATP synthase and NADH: ubiquinone oxidoreductase, involved in oxidative phosphorylation. O-EVs increase respiratory complex protein expression, mitochondrial density, and mitochondrial respiration in tumor cells. Mitochondrial complex I inhibitor, metformin, reverses O-EV-induced cell proliferation. Several miRNAs, miR-155-5p, miR-10a-3p, and miR-30a-3p, which promote mitochondrial respiration and proliferation, are enriched in O-EVs relative to EVs from lean women. O-EV-induced proliferation and mitochondrial activity are associated with stimulation of the Akt/mTOR / P70S6K pathway and are reversed upon silencing of P70S6K. This study reveals a new facet of the obesity-breast cancer link with human breast adipose tissuederived EVs causing the metabolic reprogramming of ER+ breast cancer cells

08

SMAD3-mediated activation of tumor-associated fibroblasts enhances cancer cell invasion in lung adenocarcinoma: potential implications in early dissemination

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Lung cancer is the leading cause of cancer death worldwide, and lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are their main histologic subtypes. Furthermore, there is clinical evidence that ADC tumors tend to disseminate earlier than SCC tumors, specially to the brain, although the underlying mechanisms remain unknown. Notably, we described the selective epigenetic repression of the pro-fibrotic transcription factor SMAD3 in SCC-TAFs, leading to reduced SMAD3 activity that was compensated by increased expression and activity of its closely related homolog SMAD2 (Ikemori et al, Cancer Res 2020). For this purpose, we formed 3D tumor spheroids by mixing fluorescently labelled ADC cancer cells and fibroblasts within a mixture of collagen and Matrigel. First, we analyzed the impact of altered SMAD2/3 expression in fibroblasts in leading CCI by knocking-down either SMAD2 or SMAD3 by shRNA in primary pulmonary fibroblasts, and used them as ADC-like or SCC-like TAF models, respectively. Confocal microscopy revealed more invasive events in tumor spheroids containing shSMAD2 (ADC-like) fibroblasts compared to shSMAD3 (SCC-like) fibroblasts. Consistently, enhanced CCI was observed in tumor spheroids bearing ADC-TAFs compared to SCC-TAFs. In agreement with our *in vitro* findings, tumor xenografts bearing shSMAD2 (ADC-like) fibroblasts mixed with cancer cells exhibited larger tumor growth and more invasive structures compared to tumors bearing shSMAD3 (SCC-like) fibroblasts. Mechanistically, shSMAD2 fibroblasts displayed higher N-cadherin expression, previously linked to collective cancer cell invasion via enhanced force transmission by TAFs (Labernadie et al, Nat Cell Biol 2017), supporting that the high SMAD3 conditions of ADCTAFs may promote CCI through by upregulating N-cadherin. Overall, our results demonstrate that SMAD3 in fibroblasts drives tumor growth and invasion of ADC cells, potentially contributing to the early dissemination observed in ADC patients.

Awarded

09

Deciphering metabolic impact of chemotherapy in colorectal cancer metastasis

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Colorectal cancer (CRC) is one of the world-leading causes of cancer-related mortality. Many patients treated with adjuvant chemotherapy present with distant recurrence and resistance to treatment. Better understanding factors influencing patient relapse and metastasis formation upon adjuvant chemotherapy is an important unmet clinical need.

In this project, we aim at deciphering the systemic effect of chemotherapy-induced injuries of non-tumoral tissues and their impact on metastatic relapse and treatment responses of colorectal tumors.

We used *in vivo* mouse model of colorectal cancer liver metastasis. Pretreatment of mice with FOLFOX durably prevented liver metastasis formation, even when cells were implanted after drug clearance. Such chemotherapy memory effect was gut microbiome-dependent. We identified a microbial derived metabolite which limited growth of human CRC tumoroids *in vitro* and CRC liver metastasis in mice.

Current work focuses on determining the impact of chemotherapy on gut microbiome and the cell-autonomous and microenvironmental mechanisms of the metastasis inhibition by gut-derived bacterial products. Our goal is to identify new therapeutic agents that could be used as potentiate existing cancer therapies.

POSTER SESSION

10

The microenvironmental heterogeneity and noninvasive oximetric imaging of glioblastoma multiforme as prognostic markers of tumor invasiveness

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Glioblastoma multiforme (GBM) is one of the most common intracranial tumors, with a high degree of malignancy and aggressiveness. One of the main reasons for the poor response to cancer treatment may be the specific microenvironment of tumors. A closer look at the molecular mechanisms and individual components of the extracellular matrix will allow for a more detailed understanding of the structure of tumors and relationship with invasiveness and metastasis. Tumor oxygenation is of great prognostic importance, so non-invasive methods of measuring the partial pressure of oxygen (pO2) in tumors may have great potential in assessing the effectiveness of anticancer therapies.

The aim of the study was to determine the degree of invasiveness, metastasis, migration and the level of adhesion proteins in glioblastoma cell lines in 2D and 3D models. An important aspect of our research was to stabilize the long-term culture by transferring spheroids from a hanging drop to plates coated with 2.5% polyHEMA or to the Clinostar™ system. Spheroids from the GL261 and U87 lines show an increased degree of invasiveness, which is manifested by the development of invadopodia in Geltrex®. We have classified the spheroids in terms of their 3D architecture and correlated the structure of spheroids with their aggressive and invasive properties. Mapping partial pressure in GBM tumors and assessment of the evolution of intratumoral pO2 heterogeneity in orthotopic and ectopic models were assessed by non-invasive electron paramagnetic resonance imaging using LiNc-BuO-based microspheres probe.

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Investigating the roles of IL13RA1 and IL13RA2 in tumor growth and metastasis

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The cytokines IL4 and IL13 are overexpressed by many human solid tumors and are associated with invasive and metastatic phenotypes. These cytokines signal through type I (IL4 only) and type II (IL4/IL13) IL4 receptors (IL4R). IL13 also signals through an alternate receptor, IL13RA2. Expression of type II IL4R correlates with poor patient prognosis in TNBC, and HER2+ tumors display elevated type II IL4R expression. Additionally, IL13RA2 is highly expressed in primary brain tumors.
We are investigating the potential roles of IL13RA1 and IL13RA2 in breast cancer growth and metastasis, with a particular emphasis on breast-to-brain metastasis. The brain-tropic TNBC cell line MDA-231-BrM2-831 (231-BrM2) demonstrates changes in the relative abundance of IL4R subunits as compared to parental MDAMB-
231, including a substantial elevation of IL13RA2 at both the message and protein level. 231-BrM2 also show increased induction of pStat6 in response to IL4 stimulation, elevated MMP1 and MMP9 expression, and preliminarily appear more migratory at baseline. Interestingly, treatment with the IL13RA2 ligand CHI3L1 decreases migration of 231-BrM2, but not of the parental cell line.
Preliminary *in vivo* data from both pharmacologic inhibition of type II IL4R and an IL13RA1 CRISPR knock-out suggest that inhibition of type II IL4R signaling reduces brain metastatic growth following intracardiac injection. Conversely, both IL13RA1 and IL13RA2 knock-out 4T1 cells show improved survival and increased proliferation compared to control in standard and low-nutrient growth conditions *in vitro*.
These data suggest that signaling through both IL13RA1 and IL13RA2 may influence tumor growth and metastasis. Previous work has suggested that loss of either receptor impairs tumor growth, which runs contrary to our observations. Going forward, we will be investigating tumor growth at multiple organ sites *in vivo* as well as probing the mechanism behind this unexpected growth and survival advantage.

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Mechanisms of Resistance to CDK4/6 Inhibitors in Advanced Breast Cancer

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Cyclin D-CDK4/6 axis of cell cycle is constitutively activated in many cancers and drives uncontrolled cell proliferation, being thus considered an attractive therapeutic target. The development of CDK4/6 inhibitors (CDK4/6i – Palbociclib, Abemaciclib and Ribociclib) and their subsequent approval in combination with hormonotherapy has transformed the treatment of estrogen receptor-positive (ER+) and human epidermal growth factor receptor 2 negative (HER2-) metastatic breast cancer (BC) due to their outstanding effects in increasing progression-free survival, controlling tumor growth and low toxicity. However, the efficacy of this strategy is still limited by the appearance of resistance to these drugs. Therefore, there is a clinical need to identify the molecular pathways responsible for the development of resistance and design new therapeutic combinations that could benefit patients in this scenario.
The aim of this project is to generate a platform to elucidate and overcome the mechanisms of resistance to CDK4/6i using both *in vitro* and *in vivo* approaches. Regarding the *in vitro* part, we have generated ER+ HER2- BC cell lines resistant to high concentrations of Palbociclib or Abemaciclib, that have been extensively characterized using a variety of techniques. Moreover, we have designed dense CRISPR/Cas9 knock-out libraries targeting the CDK, CDK-like and Cyclin families that, after being applied to the BC resistant cell lines, will help us to elucidate which is the next kinase that controls cell proliferation in the context of resistance to CDK4/6i. Once the kinase is identified, its role will be also evaluated *in vivo*, thanks to the use of different breast metastasis mouse models. This strategy offers the possibility of designing and testing the next therapeutic option for BC patients that have progressed on CDK4/6i.

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Protein homeostasis for the control of prostate cancer Metastasis

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The molecular alterations that underlay the metastatic process are a timely subject of investigation due to their potential impact on cancer-related mortality. In order to identify key pathways in the metastatic process, we made use of publically available Prostate Cancer databases using Cancertool. Through a bioinformatics approach, we found that UFMylation is altered in metastatic prostate cancer. UFMylation is one of the least understood ubiquitin-like post-translational modifications. These modifications may have the ability to alter the characteristic biological processes of their target proteins, including their stability and/or function.

In order to elucidate the role of UFMylation in prostate cancer aggressiveness, we developed molecular tools to perturb the UFMylation pathway. We observed that the silencing of UFMylation increases invasiveness at the expense of proliferation both in *in vitro* and in *in vivo* metastatic assays, a switch that has been previously linked to cancer cell dissemination. Mechanistically, we exploited the strong biotinstreptavidin binding affinity to uncover UFMylation-regultated proteins. TurboIDbased UFM1 interactome, together with the identification of proteins that are modified by UFM1 (using BioUFM1), revealed core metabolic proteins to be UFMylated. The UFMtylation of such proteins seem to alter cell metabolism by inducing protein glycosilation. Altogether, our results reveal that protein UFMylation could operate as a checkpoint to control prostate cancer aggressiveness by modulating cell metabolism.

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Lung fibroblasts regulate disseminated tumor cells fate and progression to metastasis through TFGß1/Neuropilin 2 and FGF5/FGFR2 pathways

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Metastasis is the major cause of cancer death and represents one of the major challenges in oncology. Scientists are still trying to understand the biological basis underlying the dissemination and outgrowth of disseminated tumor cells (DTCs) at secondary organs. Therefore, understanding the biology of DTCs is essential to develop better anti-metastatic therapies. Our previous studies have shown that specific microenvironment-driven mechanisms control the growth arrest and survival of dormant DTCs. We have identified the neurogene neuropilin 2 (NRP2) as a poor prognosis indicator in patients with breast and Head and Neck cancer. Our results show that the NRP2 positively regulates breast and head and neck cancer cells proliferation, migration, invasion and survival. Interestingly, lung DTCs overexpress NRP2. Moreover, NRP2 deletion inhibits tumor growth *in vivo* as well as decreases the number and size of lung metastases by reprogramming lung DTCs into a quiescent state through p27 inhibition. Furthermore, we have shown that the lung microenvironment up-regulates NRP2 expression through fibroblasts secretion of TGFβ1. In patients, NRP2 overexpression correlates with extracellular matrix remodeling pathways. In addition, treatment with lung fibroblasts conditioned medium increases lung DTCs derived cells proliferation *in vivo*. Moreover, we have previously shown that FGFR2 and FGF5 regulate fibroblasts mediated treatment resistance in breast cancer. Interestingly lung DTCs derived cell lines, upregulate FGFR2. Accordingly, FGFR2 inhibition reduces lung DTCs derived cell lines proliferation *in vivo* and induces cell cycle arrest. We conclude that lung fibroblasts promote lung metastasis through TGF-ß1 upregulation of NRP2 that induces DTCs escape from dormancy. Moreover, we hypothesize that FGF5/FGFR2 could also play a role DTCs regulation by the lung stroma.

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Oncostatin M cytokine signalling promotes breast cancer metastasis by remodelling the extracellular matrix

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Chronic inflammation is a canonical cancer hallmark and a major driver for tumour progression and metastasis. Cytokines are the main regulators of inflammation. They are secreted by immune, cancer and stromal cells, have cancer-cell intrinsic effects and modulate immune response by acting on the tumour microenvironment (TME). We recently identified the cytokine Oncostatin M (OSM) as central node for multicellular interactions between immune and non-immune stromal cells and the epithelial cancer cell compartment (1). Our unpublished data show that activation of the oncostatin M receptor (OSMR) by OSM promotes breast cancer metastasis by acting on cancer cells and cancer associated fibroblasts (CAFs) in preclinical models of oestrogen receptor (ER) negative breast cancer, including orthotopic and tail vein injections of cancer cells in immunodeficient and syngeneic mice. In the clinical setting, OSM signalling pathway is increased in the ER- subtype, where it associates with worse patient prognosis. Based on high-throughput transcriptomic analysis, proteomics and functional studies, our results support that OSM promotes breast cancer progression by promoting collagen deposition and ECM remodelling through activation of integrin-alpha 5. In addition, OSM reprograms cancer cell and CAF's metabolism to a more glycolytic and less oxidative metabolism and promotes lactate secretion and hypoxic signalling.

Collectively, our data support that the cytokine OSM:OSMR axis reprograms the immune and non-immune microenvironment and plays a key role in breast cancer progression and metastasis. Targeting OSM:OSMR interactions with blocking antibodies is a potential therapeutic strategy to inhibit tumour-promoting inflammation in ER- breast cancer.

Other Information:

Araujo AM, Abaurrea A, Azcoaga P, at al., Caffarel MM* and Lawrie C. Stromal Oncostatin M cytokine promotes breast cancer progression by reprograming the tumor microenvironment. J Clin Invest. 2022;132(7):e148667. (*sole corresponding author), PMID: 35192545

POSTER SESSION

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Are mutational patterns determining the metastatic homing?

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Introduction

According to the seed and soil hypothesis, the organ-specificity of metastatic tumors is not a random process and depends on multiple factors. Here, under the hypothesis that genetic background is also involved, we characterized the mutational and neoantigenic landscape of a cohort of primary and metastatic samples and correlated it with metastatic homing.

Methods

Mutational data from nine types of cancer including primary and metastatic samples from 19827 patients (including colorectal, breast, lung, melanoma, endometrial, bladder, ovarian, pancreas and prostate) were download from a pan-cancer cohort study (Nguyen et al, Cell, 2022). Mutational patterns across samples using recurrently altered genes (at least in 5% of patients) were extracted.

Results and discussion

As expected, PCA grouped metastatic samples mostly according to their primary site of origin no matter the target organ. Gastrointestinal tumors (colorectal and pancreatic) cluster together suggesting a similar mutational pattern. But, when analyzed separately, interesting features emerged in each kind of tumor. For example, in colorectal scenario, KRAS was more frequently mutated in lung than in liver metastases. In melanoma, lung metastases showed a higher mutational rate than tumors in other locations. However, mutations characterizing a metastatic location, regardless of their primary site of origin, were not found. In concordance, no neoantigenic pattern specific from metastatic location was found. Nevertheless, brain and lung metastases exhibited a higher number of neoantigens than others such as bone metastases.

Conclusions

Mutational patterns in metastasis were mainly determined by their primary tumor of origin. However, when tumors types were analysed separately, some trends emerged. Brain and lung metastases had a higher number of neoantigens than metastastic tumors in other locations.

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Negative regulation of SH2B3 expression by the histone methyltransferase SMYD5 in lung cancer metastasis

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The main cause of death in lung cancer patients is metastasis. Thus, efforts to suppress micrometastasis or distant metastasis in lung cancer, identify therapeutic targets and develop related drugs are ongoing. In this study, we identified SMYD5 as a novel metastasis regulator in lung cancer and observed overexpression of SMYD5 in lung cancer based on both RNA-sequencing (RNA-seq) analysis results derived from the TCGA portal and immunohistochemical analysis results; knockdown of SMYD5 inhibited cell migration and invasion by changing EMT (Epithelial-Mesenchymal Transition) marker and MMP9 expression in NCI-H1299 and H1703 cell lines. Additionally, SMYD5 knockdown increased SH2B3 expression by decreasing the level of H4K20 trimethylation. Furthermore, in an *in vitro* EMT system using TGF-β treatment, SMYD5 knockdown resulted in reduced cell migration and invasion in the highly invasive NCI-H1299 and H1703 cell lines. Based on these findings, we propose that SMYD5 could serve as a potential therapeutic target for lung cancer treatment and that cotreatment with a SMYD5 inhibitor and chemotherapy may enhance the therapeutic effect of lung cancer treatment

POSTER SESSION

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SOX11 as a potential driver of de novo metastatic Hormone-Sensitive Prostate Cancer

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Metastatic prostate cancer represents around 5-10% of the prostate cancer (PCa) diagnoses, but accounts for 50% of the deaths attributed to PCa. When apparent at diagnostic (de novo), the metastatic PCa has spread beyond the prostate gland to distant organs, but remains sensitive to hormone therapy due to not having received any prior systemic therapy. Therefore, further research into metastatic hormone sensitive PCa (mHSPC) is needed to elucidate the molecular characteristics of this form of the disease, which would answer important clinical questions and could be extrapolated to other aggressive forms of PCa.

An RNAseq analysis performed on primary tumour samples of localized and de novo mHSPC patients revealed dramatically different transcriptomic landscapes between conditions, with more than 5000 differentially expressed genes. In the case of mHSPC, processes linked to neural signalling, development and extracellular matrix organisation appeared enriched, with the POU and SOX families of transcription factors being identified as potential drivers of this phenotype. We identified SOX11, a transcription factor with a role in embryonic neurogenesis and tissue remodelling, as having an increased expression in mHSPC. Here, we demonstrate how SOX11 promotes a more metastatic phenotype in prostate cancer cells both *in vitro* and *in vivo*.

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Tumor-associated fibroblasts enhance angiogenesis in lung adenocarcinoma through TIMP-1 and VEGF-A: potential implications in early dissemination and immunosuppression

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Lung cancer is the leading cause of cancer-related deaths worldwide being the main histologic subtypes: adenocarcinoma (ADC) and squamous cell carcinoma (SCC). The fibrotic tumor microenvironment, enriched with tumor-associated fibroblasts (TAFs), plays a crucial role in tumor progression. Previously we revealed that TAFs in ADC exhibit enhanced fibrosis compared to SCC due to the epigenetic repression of SMAD3 in SCC-TAFs. Moreover, they respond differently to Nintedanib and other antiangiogenic drugs, suggesting a dependency on the histologic subtype for angiogenesis. There is a renewed interest in using antiangiogenic therapies to enhance the response to immune checkpoint inhibitors. Nevertheless, the role of lung TAFs in the promotion of angiogenesis and the resistance to anti-angiogenic therapies remains poorly defined. The pro-angiogenic function of the conditioned medium (CM) of TAFs from ADC and SCC patients was analyzed using migration and network formation assays of endothelial cells. The secretion of pro-angiogenic factors in TAFs was analyzed using an angiogenesis array. Selected factors were functionally validated *in vitro* and *in vivo* using genetic models. All angiogenesis markers were upregulated in ADC compared to SCC concomitantly with a lower necrosis in ADC, which correlates with the clinical observation that ADC patients exhibit metastasis earlier than SCC. The CM of ADC-TAFs elicited larger angiogenesis than SCC-TAFs. The pro-angiogenic factors TIMP-1 and VEGF-A were overexpressed in ADC-TAFs. The knocking down of TIMP-1 by siRNA in ADC-TAFs impaired angiogenesis *in vitro* and in tumor xenografts *in vivo*. Our results reveal a larger angiogenesis in ADC than SCC tumors and implicate the TGF-β1/SMAD3/TIMP-1 pathway in the induction of angiogenesis of ADC-TAFs and provide a rationale for the earlier metastasis of ADC patients based on the enhanced pro-angiogenic role of ADC-TAFs.

POSTER SESSION

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Deciphering cellular plasticity of metastatic cancer cells upon cholesterol-pathway inhibition

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Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic fatal malignancy, yet the metastatic process is still poorly understood. During metastasis or upon drug treatment the cancer cells show a high cellular plasticity to develop therapy resistance or to adapt to changes in their respective environment. Statins are among the most commonly prescribed drugs and known as inducers of EMT (epithelial to mesenchymal transition) and modulators of cellular plasticity in PDAC (Dorsch et al., Cell reports 2021). We discovered that the response to the statinenforced switch to a more mesenchymal-like cell state depends on the cancer cells' ability to activate ERK signaling, where the cancer cells either become apoptotic, or escape apoptosis if they can activate ERK. Yet, ERK activation is not mediating the phenotypic switch itself. We have characterized multiple human PDAC cell lines for their morphology, metastatic seeding ability, and their capability to activate ERK and escape apoptosis upon statin treatment. Proteome analysis of cells with different levels of plasticity discovered several upregulated proteins involved in non-coding RNA processing pathways, a process that is known to influence proliferation of cancer cells, suppress apoptotic pathways, increase metastatic potential, and acquiring of drug resistance. Further *in vitro* and *in vivo* molecular analyses will provide valuable insights into the regulation of cell plasticity as this is detrimental for successful therapy of advanced or metastatic disease stages and a major reason for therapy escape. It is of utmost clinical importance to understand this effect to open up new therapeutic avenues.

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Dynamic deposition of histone H3.3 is a major regulator of dormancy

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Metastases represent the leading cause of breast cancer mortality. Dormancy is one of the most critical, yet poorly understood, steps of the metastatic process during which cancer cells persist in a non-proliferative state for extended period of time despite therapy. The reawakening of dormant breast cancer cells (DCCs) often confers metastatic disease resistance to standard-of-care therapies. Therefore, DCCs pose a significant clinical challenge in breast cancer patient care. Chromatin remodeling serves as a foundation for the cellular reprogramming required to drive cell fate transitions. However, little is known about the nature of this remodeling within the context of dormancy. Here, we focus on nucleosome composition as a key regulator of DCCs' cell fate throughout the metastatic cascade. We hypothesize that differential deposition of histone H3.3 driven by its cognate chaperone, the HIRA complex, determines whether DDCs enter and remain in dormancy or proliferate and form overt metastases. Our data demonstrates that suppression of histone H3.3 deposition onto chromatin leads to cell cycle arrest in G0/G1, inhibition of proliferation and activation of canonical dormancy signaling, in a reversible manner. Mechanistically, we reveal that suppression of H3.3 directly blocks the transcription of SKP2, the main regulator of p27 degradation, resulting in the accumulation of p27 and cell cycle arrest in G0/G1. Lastly, we demonstrate that this regulatory mechanism is physiologically relevant, occurring in wellestablished isogenic models of dormancy and driven by the regulation of HIRA in DCCs by dormancy-regulating factors of the TME. Collectively, our work unveils an uncharacterized role for H3.3-HIRA axis of chromatin remodeling as a major regulatory point of DCCs' cell fate by controlling their transition between dormant and proliferative states and puts forward HIRA as a therapeutic target to prevent reawakening of DCCs and halt metastatic disease development.

Awarded

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MAF amplification promotes bone metastasis in ER+ breast cancer

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Breast cancer (BCa) is the most diagnosed cancer in women and metastasis is the leading cause of death in BCa patients, being the bone the most preferred metastatic site for BCa cells. The discovery of MAF amplification, which occurs in 20% of these patients, and its prognostic value for estrogen receptor + (ER+) BCa patients at risk of bone relapse opened the door to study how this AP-1 transcription factor facilitates and mediates bone colonization of BCa cells.
Using human well-established ER+ BCa cell lines, we noted that the sole overexpression of MAF is sufficient to substantially increase bone metastasis rates, with no differences in metastasis to other sites; which was also validated using ER+ tumor-derived cell lines (named as mTB cells) from a novel Maf overexpression knock-in mouse model. Moreover, when we implanted control and MAFoverexpressing cells in the mammary fat pad, we observed no variations in the presence of circulating tumor cells, indicating that MAF plays an important role in the later stages of the metastatic process. Subsequently, we explored the bone metastasis selectivity of MAF-amplified cells by co-injecting MAF+ and MAF- mTB cells into the mouse tibia, and we identified that MAF-expressing cells lead and are essential for bone colonization process.
In light of the fact that MAF-amplified BCa patients show a poor response to adjuvant treatment with bisphosphonates (which can prevent bone metastasis and improve both DFS and OS, but only in patients with non-amplified MAF breast cancer), we delivered MAF+ mTB cells intracardially to immunocompetent mice and treated them with zoledronic acid. Our findings revealed that there was a significant increase in extra-skeletal metastasis, mostly in lung, liver, kidney, ovarian and brain, as described in patients.

23 Dissecting the Influence of Tumor-Associated Fibroblasts in HER2+ Breast Cancer Lung Metastasis

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Breast cancer (BC) is the leading cause of cancer death among women. HER2+ tumors account for 20% of all BC having aggressive clinical course. The presence of disseminated tumor cells (DTCs) at early stages is associated with higher risk of recurrence. Therefore, DTCs characterization is required to identify new therapeutic targets for their metastatic dissemination. This study aimed to investigate the influence of tumor-associated fibroblasts (TAFs), the predominant cell type in the tumor microenvironment, on HER2+ BC dissemination to the lung, a major HER2+ BC metastatic site.

To examine the crosstalk between HER2+ BC cells and TAFs at different stages, we obtained primary fibroblasts from either HER2+ BC primary tumors or lungs,. We also generated resistant cell lines to HER2 targeted therapies *in vitro* (HER2-R), and HER2+ breast cancer cells primed for lung metastasis (HER2-Lu) , by inoculating them into nude mice and isolating those that have colonized the lung after three months. Our previous work identified and validated the FGF5/FGFR2 axis as a novel mechanism of resistance to anti-HER2 therapies throughFGF5 secretion in TAFs and FGFR2 activation in HER2+ BC cells. HER2-R cells also exhibited an overexpression of dormancy markers like DEC2. Notably, *in vivo* experiments revealed higher FGFR2 pathway expression in HER2-Lu cell lines from lung metastases compared to primary tumors, supporting that metastatic cells might be dependent on the same pathways than resistant cells for survival. Moreover, metastatic FGFR2high BC cells showed a significant increase in circulation survival by using a novel circulation-on-a-chip-model.

Our results implicate TAFs in HER2+ BC cell dormancy, therapy resistance, circulation survival and lung metastasis. Our findings support that targeting this axis could offer a promising therapeutic approach to eradicate dormant DTCs as well as DTCs survival in circulation, and ultimately prevent HER2+ BC metastasis and clinical relapse.

24 Antagonist roles of Midkine in melanomagenesis: Timing and Location matters

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Melanomas are well known to progress with large changes in the transcriptome of both, melanoma cells and the tumor microenvironment. Curiously, oncogenic signals (in melanomas and other cancer types), that favor tumor development at advanced stages of the disease may have inhibitory functions at earlier time points. The contribution of the stroma to these seemingly opposing features is unclear. Factors that control this switch are not well understood. Midkine (MDK) is a growth factor identified in melanoma as a secreted driver of metastasis (Olmeda et al. Nature, 2017; Cerezo-Wallis et al. Nat Med, 2020). However, the specific contribution of MDK to early stages of melanoma initiation is unknown. Here, we will present histological analyses of MDK in primary melanomas aided by artificial intelligence. These studies were complemented with 3D *in vitro* systems and genetically-engineered melanoma mouse models that together, support an antagonist role of non-tumoral Midkine from epidermal and mesenchymal cell populations during melanomagenesis. These studies provide new insight on the impact of the tumoral stroma in shaping the ultimate outcome of tumor-secreted factors in melanoma initiation and progression.

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Deciphering the role of NGFR in head and neck squamous cell carcinoma metastasis and resistance to therapy

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Head and neck squamous cell carcinoma (HNSCC) ranks as the most prevalent cancer worldwide. While early-stage HNSCC is curable, two-thirds of patients are diagnosed at advanced stages where chemotherapy often fails. The expression of nerve growth factor receptor (NGFR) in HNSCC tumor cells is a poor prognostic marker linked to stem-like features. However, its role in therapy resistance remains unclear. This project aims to elucidate the significance of NGFR in tumor development, metastasis and chemotherapy resistance.

We confirmed NGFR expression in the murine MOC2p and the human FaDu and VDH15 cell lines. Moreover, we studied the role of Ngfr by knocking out its expression in the MOC2p model and evaluated the *in vivo* effect after orthotopic injection. Interestingly, we observed a reduction in tumor growth and metastasis in mice injected with Ngfr-KO. To understand the mechanism involved, we performed an RNA-seq of MOC2p Ngfr-KO tumors. We obtained 51 upregulated and 30 downregulated genes in the Ngfr-KO. GSEA showed the modulation of pathways relevant in cancer; we are currently analyzing these findings. In addition, we characterized NGFR expression in a cohort of 339 tumors from HNSCC patients. High NGFR expression (p-value=0.026) and the diffuse expression pattern (pvalue 0.036) were associated with lymph node metastasis. Moreover, the diffuse pattern was linked to distant metastasis (p-value=0.003). High NGFR also positively correlated with poor response markers (e.g. active SRC or nuclear β-catenin) and negatively with good prognosis markers (e.g. pS6240 or p21).

For the analysis of NGFR in therapy resistance, we are characterizing patientderived organoids and chemotherapy-resistant tumors. NGFR was highly expressed in a chemotherapy-resistant organoid and in xenografts derived from chemotherapy-resistant cells. These findings underscore the significance of NGFR in HNSCC, proposing it as a critical factor in tumor development and resistance to chemotherapy.

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Silencing the glycerophosphodiesterase EDI3 reduces breast cancer metastasis in experimental and spontaneous mouse models

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Despite advances in cancer treatment, metastasis remains a major problem for tumor therapy. In endometrial and ovarian cancer, the glycerophosphodiesterase EDI3 (endometrial carcinoma differential 3, GPCPD1) was found to be associated with metastasis and worse survival. EDI3 is a key enzyme that links choline and glycerophospholipid metabolism by hydrolyzing glycerophosphocholine (GPC) to produce choline (Cho) and glycerol-3-phosphate (G3P). Altered choline metabolism has been reported in several malignancies, including breast cancer. Recent work on EDI3 reported high expression in ER-HER2+ breast tumors and cell lines, and silencing EDI3 was shown to reduce viability in these cells.

In order to elucidate EDI3's role in metastasis, we established a doxycyclineinducible knockdown system in luciferase-expressing ER-HER2+ breast cancer cells (HCC1954-luc) to study how silencing EDI3 influences processes relevant for metastasis *in vitro* as well as metastasis formation *in vivo*. For this purpose, we performed various cell assays and used mouse models of both experimental and spontaneous metastasis.

Our studies showed that doxycycline-induced EDI3 silencing resulted in altered phospholipid metabolite levels, as well as reduced colony formation, adhesion, proliferation and resistance to anoikis. Bioluminescence imaging revealed lower signals in the lung and peritoneal cavity of mice injected with cells expressing less EDI3 after tail vein and intraperitoneal injection, respectively, indicative of reduced metastasis. Furthermore, mice injected with cells expressing reduced levels of EDI3 also developed less ascites and had a higher probability of survival. After orthotopic injection both primary tumor growth in the mammary fat pad of mice and metastatic spread to the lungs was reduced when EDI3 was silenced.

The obtained results suggest that silencing EDI3 leads to reduced metastatic burden, making it a potential treatment target in metastasizing ER-HER2+ breast tumors.

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RANK Signaling and Metabolic Rewiring in Postmenopausal Breast Cancer

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Despite strong preclinical data, the therapeutic benefit of the RANKL inhibitor, denosumab, in breast cancer patients, beyond the bone, is unclear. Aiming to select patients who may benefit from denosumab, we analyzed RANK protein expression in more than 2,000 breast tumors from four independent cohorts. Intriguingly, tumor RANK protein expression associated with poor prognosis in postmenopausal breast cancer patients, activation of NFKB signaling and modulation of immune and metabolic pathways. We hypothesize that activation of RANK signaling increases in breast cancer from postmenopausal women and promotes tumor aggressiveness by modulating tumor metabolism.
We aim to characterize the contribution of tumor RANK expression to the metabolic changes imposed by menopause and its influence on tumor outcome and therapy response. We found that Rank overexpressing tumors grow faster and seed more metastasis when implanted in overiectomized mice. Molecular and metabolic characterization of systemic and intratumoral changes is ongoing.
This study holds the potential to elucidate how RANK signalling shapes breast cancer metabolism, particularly within the context of a patient's hormonal status. The implications of our work extend to the clinical field, with approved RANK inhibitors at our disposal and the possibility of uncovering new metabolic vulnerabilities for therapeutic targeting.

POSTER SESSION

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Metastasis

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The tumor interstitial fluid of metastatic lungs is enriched with the actin-binding proteins Scinderin and Fascin1

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Isolation of tumor interstitial fluid (TIF) directly from tumors is a powerful method to characterize the tumor microenvironment. To analyze the metastatic nodules microenvironment, we used a mouse model that provides a sufficient volume of TIF bathes the metastatic lungs. In SCID mice, we retro-orbitally injected phosphate saline buffer (PBS) as a control or rat prostate tumor cells (R3327-5'A). These cells produce a large number of lung nodules in a short period of time. Fifteen days after injection, the lungs were excised and TIFs obtained by centrifugation (Matas-Nadal et al, Mol Cell Prot. 22:100547 2023). TIF samples from metastatic lungs (R3327-5'A; n=5) and normal lungs (PBS; n=5) were analyzed by label-free quantitative proteomics to obtain protein profiles. Comparison between metastatic and normal lungs yielded a list of 37 proteins significantly up-regulated in metastatic lung TIFs. A GO analysis revealed that this list, was enriched in cytoskeletal proteins such as the actin-binding factors Scinderin (SCIN) and Fascin 1 (FSCN1).
We have confirmed the expression of these proteins in mouse lung nodules and their TIF by immunohistochemistry and immunoblot. We have also detected by conditioned media analysis (secretome) that R3327-5'A cells secrete SCIN and FSCN1. The relevance of these cytoskeleton proteins in TIF and their role in the metastatic response is our subject of study.

POSTER SESSION

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PRMT7: an arginine methyl-transferase that enhances the progression of localized prostate cancer to metastasis

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Metastatic prostate cancer patients (mPCa) have a reduced survival rate in comparison to patients with localized tumors. It is mainly due to the inefficacy of available treatments to manage advanced or metastatic stage of the disease. Therefore, it is crucial to find new therapeutic targets to increase mPCa patient survival. To do so, we sought to identify the most relevant regulators of mPCa onset by performing two independent high-throughput CRISPR/Cas9 screenings in PC-3 and DU 145 highly metastatic prostate cancer cell lines. Several of our best screening hits were validated using small interference RNA (siRNA) technology, being PRMT7 our best candidate. Moreover, we demonstrated that its inhibition via siRNA, pharmacological inhibitors or its depletion via CRISPR/Cas9, reduces mPCa cell invasive, migratory and proliferative capacities. Importantly, we further confirmed that PRMT7 depletion reduced PCa metastasis appearance in *in vivo* models of chicken chorioallantoic membrane (CAM) and mouse xenograft assays. Additionally, we found that this gene is upregulated in primary tumor samples of patients at advanced stages in a Spanish PCa samples cohort. Lastly, our differential RNA-seq transcriptomic analysis suggested that PRMT7 could promote mPCa appearance by, reprograming the expression of several adhesion molecules through methylation of several transcription factors, such as FoxK1 or NR1H2, that result in primary tumor cell adhesion loss and motility gain. Thus, we propose PRMT7 as a potential therapeutic target and biomarker for mPCa.

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The EDN1/EDNRA/ß-arrestin axis promotes colorectal cancer progression by regulating STAT3 phosphorylation

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Endothelin receptor A (EDNRA) has been reported to play various crucial physiological roles and has been shown to be associated with the pathology of several diseases, including colorectal cancer (CRC). However, the molecular mechanisms of EDNRA in the development of human CRC have not been fully elucidated to date. In this context, the present study was performed to investigate biological functions and novel downstream signaling pathways affected by EDNRA, during CRC progression. First, using public data repositories, it was observed that the EDRNA expression levels were markedly increased in CRC tissues, as compared to normal tissues. Patients with CRC with an increased EDNRA expression exhibited a significantly decreased survival rate in comparison with those with a lower EDNRA expression. The ectopic expression of EDNRA or its ligand, EDN1, promoted, whereas the silencing of EDNRA or EDN1 decreased cell proliferation and migration *in vitro*. To elucidate the signaling pathways involved in the regulation of EDNRA expression in CRC cells, a phosphokinase array analysis was performed, and it was observed that the knockdown of EDNRA substantially suppressed the phosphorylation of STAT3 in CRC cells. Of note, STAT3 silencing simultaneously decreased EDN1 and EDNRA expression, with the expression of EDN1 and/or EDNRA appearing to be directly regulated by binding STAT3 to their promoter region, according to chromatin immunoprecipitation and promoter assays, ultimately indicating a positive feedback loop in the expression of EDNRA and EDN1. It was also observed that treatment with an EDNRA antagonist (macitentan), alone or in combination with cisplatin, suppressed cell growth and migration ability, and induced cell apoptosis. Collectively, these data suggest a critical role of the EDN1/EDNRA signaling pathway in CRC progression. Thus, the pharmacological intervention of this signaling pathway may prove to be a potential therapeutic approach for patients with CRC.

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The BASP1 signaling protein interferes with the oncogenic capacity of MYC

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MYC is a transcription factor with oncogenic potential controlling fundamental cellular processes like cell proliferation, metabolism, differentiation, or apoptosis. While MYC activities in normal cells are essential and tightly regulated, MYC is frequently found to be deregulated in most human tumors, in which this oncoprotein represents a major cancer driver.
One of the multiple transcriptional MYC targets is the brain acid-soluble protein 1 (BASP1), which is downregulated in a variety of MYC-dependent cancer cells. Recently, we found that ectopic BASP1 expression interferes with MYC-induced cell transformation, whereby BASP1 sequesters the MYC-interaction partner calmodulin (CaM) leading to MYC protein destabilization.
Using human colon cancer cells featured by high MYC expression and a silenced BASP1 Proteome comparison of SW480 cells with those ectopically expressing BASP1 (SW480-B) was performed using liquid chromatography coupled to mass spectrometry (LC MS). From 4,543 analyzed proteins, 278 were found to be specifically activated in BASP1-expressing cells including the tumor suppressor TP53. Among the 252 downregulated proteins are the MYC-associated factor X (MAX) and the metastasis-associated protein 1 (MTA1), the latter representing a known MYC target with intrinsic oncogenic activity.
Because BASP1 suppression and malignant growth inhibition by ectopic BASP1 occurs in several human tumor cell types, re-activation of the dormant BASP1 gene using the CRISPRa technology was performed in SW480 applying a lentiviral system, and cells were tested for interference with human cancer cell growth and viability. With regard to the tumor-suppressive role of BASP1 in human cancer, we also tested BASP1-mimetic peptides to develop strategies for the treatment of tumor cells featured by high MYC expression.
Other Information: Due to its pleiotropic functions in the regulation of cellular transcription, and its implications in DNA replication, chromatin remodeling, or metabolism, MYC has a fundamental impact in the control of cell growth and proliferation. Aberrant MYC activation ultimately provokes derailed cell signaling and neoplastic cell transformation in concert with additional oncogenic drivers. Therefore, pharmacological or genetic inhibition or attenuation of MYC represents a rational approach to interfere with the growth and viability of MYC-dependent human tumors.

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Epithelial to mesenchymal transition trajectories in cancer and their impact on metastasis

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Epithelial plasticity is at the core of crucial processes including embryonic cell migration, cancer progression, organ fibrosis, and tissue repair. The epithelial-tomesenchymal transition (EMT) triggers cell plasticity in all these contexts, highlighting its pleiotropy and intrinsic complexity. Seminal studies have classified EMT states in cancer cell lines and animal models. This variety of EMT phenotypes needs further investigation, particularly those relevant to the progression of prevalent and devastating diseases such as cancer.
Here, we combine lineage tracing and single-cell transcriptomics in a breast cancer mouse model to reveal different EMT programs that differ in function and are governed by different EMT transcription factors. After inferring cellular trajectories, we reconstructed the evolution of these EMT programs, unveiling a non-linear structure of the different EMT states in the tumors. In addition, multiplex labeling allowed to spatial allocation of these distinct EMT programs in mouse and human tumor samples. Finally, genetic interference with one of the trajectories showed a great impact on the invasive and metastatic potential of the tumors. Altogether, this work unveils distinct EMT trajectories in cancer, which should help propose improved therapeutic strategies for cancer.

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From Inflammation to Invasion: Unraveling Myeloid-Derived-Reactive-Oxygen- Species-SNAI Signaling in Metastasis

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Metastasis is primarily driven by the phenomenon of epithelial to mesenchymal transition (EMT), orchestrated by epigenetically regulated transcription programs within cancer cells. Recent studies suggest a connection between hypoxia-induced intracellular ROS and EMT. A direct link between extracellular ROS released by inflammatory cells on EMT not been demonstrated. Tumor-infiltrating myeloid cells are capable of producing high levels of extracellular ROS via the NOX2 enzyme and generally negatively impact on prognosis. Employing a comprehensive array of *in vitro*, *in vivo*, and in silico analyses, we illuminate the role of myeloid-cell derived ROS in initiating EMT in cancer cells, culminating in the formation of metastases.
Methods: Hydrogen peroxide was added to MCF-7, T47D, and 4T1 breast cancer cells. Then, the EMT status of these cells was assessed by RT-PCR and a wound healing assay. RNA-seq data derived from H2O2-treated MCF-7 cells and monocyte cocultured T47D cells were scrutinized with gene set enrichment. Monocytes were added to cell culture inserts within wells containing breast cancer cells together with ROS-inducing or inhibiting agents. Tumor cell wound healing in the presence or absence of monocytes, along with the expression levels of EMT-associated genes, were subsequently assessed. Alternatively, EO771 cells was co-cultured with Gr1+ myeloid cells obtained from either NOX2 knock-out or wild type Black/6 mice, and wound healing was evaluated.To evaluate impact of extracellular ROS on metastasis *in vivo*, luciferase-tagged 4T1-breast cancer cells were orthotopically implanted into the breast pads of mice, was followed by intratumoral injections of H2O2, the myeloid cell ROS-inducer D-peptide, or the NOX2-inhibitor histamine. The development of lymph node metastasis was monitored by *in vivo* imaging.
Results: Our experiments consistently showed that myeloid-cell derived ROS and H2O2 induced EMT in cancer cells via enhanced SNAI transcription family.

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Decoding the pro-metastatic role of the CoREST complex in breast cancer brain metastasis

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Metastatic spread to the central nervous system, notably brain metastasis, marks a grave progression in breast cancer, representing a substantial clinical challenge due to poor prognosis and limited treatment options. Understanding the underlying molecular mechanisms facilitating breast cancer brain metastasis (BCBM) is urgent to devise novel therapeutic strategies.

Leveraging a clinically profiled cohort of patient-matched primary breast and brain metastasis samples, we conducted RNA-seq pathway enrichment analyses and integrated transcription factor Chromatin-Immunoprecipitation (ChIP)-seq with brain metastasis transcriptome datasets. This was complemented with a highthroughput drug screen using five distinct cell line models representing all clinical subtypes to pinpoint common vulnerabilities.

Our analysis identified a pivotal role for REST and its transcriptional partners, histone-deacetylases (HDACs), in regulating brain metastasis. Elevated REST expression correlated with poor brain metastasis-specific survival. Furthermore, the HDAC pathway emerged as a top candidate from our drug screening, a finding substantiated through *in vitro* and *ex vivo* validations in patient-derived brain metastatic tumour organoids. Having identified HDAC1 as the pivotal element in patient BCBM samples, we executed CRISPR studies complemented by RNA-seq profiling. This approach elucidated key pathways prevalent in breast cancer model systems representing HER2 and TNBC subtypes. These pathways include HER/AKT /mTOR, cell cycle-related mechanisms, TGFB pathways, and metabolic and extracellular-related pathways. Current efforts are directed towards functionally verifying these identified pathways both *in vitro* and *ex vivo*.

Our findings underscore the REST/HDAC axis's potential role in promoting brain metastasis, orchestrating pro-metastatic transcriptional programs. Targeting this axis unveils a promising avenue for BCBM treatment.

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A positive feedback loop between IFN-gamma signaling and cholesterol uptake sustains PD-L1 expression in breast cancer cells

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Cholesterol uptake via binding of the low-density lipoprotein (LDL) to the LDL receptor (LDLR) has been shown to induce aggressive behaviors in breast cancer (BC) cells, including increased proliferation, intravasation, and metastasis. The regulation of LDLR expression at the tumor microenvironment is, however mostly unknown. Here we tested a small panel of cytokines, known to be present in the tumor microenvironment, for the capacity to induce LDLR expression and cholesterol uptake in (BC) cells. We show that IFN-gamma (IFNg) induces LDLR protein expression in three different BC cell lines in a JAK-STAT-1-dependent way. Moreover, treatment with IFNg increases LDL uptake and cholesterol enrichment of BC cells. These findings were somehow surprising as IFNg has mostly antitumoral functions in BC, and increased cholesterol uptake is mainly pro-tumoral.

However, a previously described pro-tumoral function of IFNg is the induction of PD-L1 expression. As such, we then tested how increased cholesterol uptake affects PD-L1 surface expression in BC cells. We observed that, surprisingly, exposing BC cells to LDL alone increases PD-L1 surface expression and, according to our hypothesis, IFNg and LDL added simultaneously, further increases PD-L1 at the cell surface – suggesting that both molecules act synergistically. Finally, we show that LDL induces IFNg receptor surface expression and downstream signaling.

Taken together, our data suggest the existence of a positive feedback loop between IFNg signaling and cholesterol uptake through LDLR, which sustains PD-L1 expression in BC cells. This increases our knowledge of how cancer cell metabolism is affected by tumor microenvironment components. It also shows that cholesterol metabolism plays a role in tumor cell interaction with the immune component of the tumor microenvironment. It further supports that controlling systemic LDL levels in patients with BC may be important for the outcome of the disease.

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Polyaneuploid Cancer Cells (PACCs) as metastasis-competent cells

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Polyanuploid Cancer Cells (PACCs) are enlarged, treatment-resistant cells with increased DNA content that form in response to stress. After stress-induction, PACCs exist in a non-dividing, but endocycling state *in vitro* and *in vivo*. PACCs are found in primary and metastatic tumors, and tumors with fewer PACCs are predictive of metastasis-free survival. We propose Polyaneuploid Cancer Cells (PACCs) play an integral role in metastasis.

Single-cell tracking revealed that PACCs are more motile than nonPACCs, have increased cytoskeletal dynamics, and exhibit increased directional migration via chemotaxis, predicting successful invasion. Several approaches revealed that PACCs overexpress Vimentin (VIM), a cytoskeletal component known to confer motility and hyper-elasticity. Biophysical assays revealed that PACCs are hyper-elastic: PACCs have increased peripheral deformability and maintained peri-nuclear cortical integrity, both of which predict successful intravasation. Functional hyper-elasticity of PACCs was confirmed using a custom microfluids device.

Ablation of VIM caused a reduction in motility, indicating a necessary role for VIM in PACC migration. Yet, lack of correlation between VIM content and motility on a per cell basis suggests that VIM is not sufficient to drive PACC migration. Assaying other sources of PACC motility, it was discovered that PACC-conditioned media confers increased migration to non-PACC cells. A cytokine array of PACCconditioned media revealed overexpression of molecules known to induce motility (IL-6, IL-8). We are currently investigating autocrine pathways that may work alongside VIM to drive PACC invasion and intravasation.

Lastly, anoikis-resistance assays and detection of PACCs in patient blood revealed that PACCs are capable of surviving in the circulation. Taken together, our *in vitro* work to date suggests PACCs are capable of invasion, intravasation and circulation: a hypothesis we are currently testing in a murine metastasis model.

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Establishment of a triple-negative breast cancer brain metastasis murine model and investigation of its metabolic signature.

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Breast cancer (BC) is the second leading cause of central nervous system (CNS) metastases, which include leptomeningeal (5% of CNS metastases) and parenchymal brain metastasis (95% of CNS metastases) [1-2]. The overexpression of human epidermal growth factor receptor 2 (HER2+) and triple-negative breast cancer (TNBC) are the most aggressive subtypes with a higher risk of developing breast cancer brain metastases (BCBM). Brain metastases (BM) occur in approximately 10-30% of patients with advanced metastatic BC and are associated with poor prognosis [2-4].

The emergence of new therapeutic strategies depends on understanding how cancer cells modify their metabolic activity, enabling them to proliferate in brain tissue. To provide information in this field, we established a murine model of brain metastasis by injecting human breast cancer cells into the brain of immunodeficient mice.

The derived cell lines generated by serial intracranial injections increase the aggressiveness of the model as the cells acquire increased proliferative competence in the brain, with a significant decrease in mouse survival (Log-rank test, $p < 0.001$).

Brain-derived cells show changes in gene expression related to cellular metabolism. The transcriptomic study revealed a significant increase in the expression of ACOX1, ACADL, CD36, CPT1A, ACOT2, FABP12, and PPARA (Student t-test, $p < 0.05$). All these genes are involved in the beta-oxidation pathway, suggesting a switch to fatty acid metabolism during tumour development in the brain parenchyma.

This metabolic modification could therefore be associated with a marked increase in the survival and proliferation of tumour cells in the brain microenvironment. To validate that adaptation of tumour cell metabolism is a key feature of BCBM progression, further investigation is required. This could lead to the development of a new approach for the treatment of BM by targeting metabolic pathways.

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A novel role of platelets to promote epithelial-mesenchymal transition in prostate cancer

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Background: Prostate cancer is one of the most common types of cancer, and the development of advanced metastatic disease leads to mortality. Tumor metastasis is a process involving the spread of tumor cells from their primary site and colonization in a secondary organ (Rebello et al., 2021). For the development of metastasis, tumor cells lose phenotype of epithelial cells and acquire mesenchymal phenotype giving them properties such as increased motility and invasive characteristics, event known as epithelial-mesenchymal transition (EMT). Metastasis depends on the interactions between tumor cells and the host microenvironment within the circulation, lymphatic vessels, and target tissues, involving blood cells (Fares et al., 2020) . Platelets are anucleated blood cells that play a crucial role in maintaining homeostasis; however, they can participate in other physiological and pathophysiological processes, such as cancer. It has been reported that platelets promote the progression of various types of cancer, such as breast, ovarian, lung, colon, and pancreatic cancer, etc (Haemmerle et al., 2018). Nevertheless, the role of platelets in prostate cancer has been poorly studied. In this work, we analyzed the role of platelets in promoting the metastatic phenotype *in vitro* model. Methods: Platelets were isolated from blood samples collected from healthy volunteer donors. Prostate cancer cell lines, LNCaP and PC-3, were exposed to platelets. The physical interaction between platelets and cell lines was evaluated by immunofluorescence assays. Epithelial-mesenchymal transition markers were evaluated by qPCR and Western Blot. Cell migration and invasion capabilities were analyzed using wound-healing assay and transwell assay. Results: Immunofluorescence revealed a physical interaction between platelets and tumor cells. In addition, exposure to platelets altered the expression of EMT markers (ZEB1, N-cadherin, Slug, Snail, Vimentin, E-cadherin, Fibronectin and Twist) in mRNA and protein cell lines. In addition, platelet treatment increased migration and invasion of tumor cells. Conclusions: Platelets have the ability to physically interact with tumor cells, promote the expression of EMT markers and induce migration and invasion in prostate cancer cell lines. Knowing the role of platelets in the progression of cancer opens the possibility of finding new therapeutic targets.

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HIVEP2 expression induces chemoresistance and tumor relapse in colorectal cancer

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Slow-cycliness is a reversible non-genetic delay in the cell cycle which allow cancer cells to survive antitumoral therapies while the vast majority of proliferating cells can be eradicated by treatments. In the clinics, this slow-cycling phenotype in cancer cells can lead to chemoresistance and relapse after an apparent remission of the disease. We developed a strategy that allows the identification and isolation of slow-cycling cancer cells (SCCCs) using the inducible expression of the histone 2B fused to the enhanced green fluorescent protein (H2BeGFP). By using gene expression microarrays we identified HIVEP2 as one of the highest overexpressed genes in SCCCs compared to rapid-cycling cancer cells (RCCCs).

Modulation of HIVEP2 expression showed its participation in the self-renewal capacity of tumor cells, as well as resistance to chemotherapies both in RCCCs and SCCCs subpopulations. Moreover, HIVEP2 overexpression causes a delay on the cell cycle by accumulating cells in the G0/G1 phases. RNAseq results confirmed that HIVEP2 regulates many pathways involved in cell differentiation and proliferation. *In vivo* experiments with HIVEP2 knock-down tumor cells are in consonance with previous results, revealing an earlier tumor initiation capacity, a slight increase in tumor volume as well as a better response under irinotecan treatment. Additionally, high expression of HIVEP2 associates with worse progression-free survival on a cohort of chemo-treated patients.

Altogether, our results propose HIVEP2 as a potential factor involved in the maintenance of the slow-cycling phenotype during carcinogenesis and a likely component to modulate its expression as a combination treatment.

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Ras drives malignancy through stem cell crosstalk with the microenvironment

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Squamous cell carcinoma (SCC) is one of the most frequent solid tumors in humans and is a major cause of cancer mortality. A group of cells located at the tumor-stroma interface called cancer stem cells (CSCs) not only have increased tumor-initiating capacity but are also resistant to chemotherapy and immunotherapy. However, the changes in CSCs during progression from a benign state to invasive SCC remain elusive. Here, we show that following HRASG12V activation, CSCs rewire their gene expression program and trigger a self-propelling, aberrant signaling crosstalk with their tissue microenvironment that triggers angiogenesis and TGFβ-signaling, creating conditions ripe for hijacking leptin and leptin receptor (LEPR)-signaling, which in turn launches downstream PI3K-AKTmTOR signaling at the benign-malignant transition.

POSTER SESSION

Brain metastases: What can mathematics teach us?

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Brain metastases (BM) are the most common intracranial tumor in adults with around 20% of cancer patients developing BMs. Stereotactic radiosurgery (SRS) is increasingly used in the treatment of BM. However, SRS can induce radiation necrosis (RN), a transient adverse event appearing after irradiation, difficult to distinguish from tumor progression and observed in 5% to 25% of treated patients. RN may resolves spontaneously, not requiring further work-up, while progression need additional treatment. Thus, distinguishing between RN and progression is clinically relevant in the management of BM.
Scaling laws (SLs) are simple mathematical models allowing to describe tumor growth. We used SLs to characterize the growth dynamics of BMs subject to different treatments and without treatment. To characterize the dynamics, a growth factor, the scaling law exponent beta, was used. There were significant differences ($p<0.005$) between patients experiencing RN and tumor progression after SRS. BMs grew faster, leading to super-exponential growth, when they developed RN. Currently, the only available criterion for assessing BM response to treatment is the one-dimensional RANO-BM criteria. We propose the introduction of a volumetric criterion, which demonstrated its ability to evaluate every lesion, regardless of its shape or size, and yields superior classification results compared to the RANO-BM criteria. Additionally, certain morphological measurements derived from routinely acquired MRIs, such as surface regularity and the extent of necrosis, were identified as predictive factors in this context.

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Defining the role of physiological aging during lung cancer progression and metastasis

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Aging is a complex biological process defined by diverse alterations in metabolism, inflammation, senescence, genomic instability, and other physiological factors that significantly impact disease development and progression. Strongly linked to cancer, aging is a key risk factor for cancer incidence and outcomes.
Of note, incidence of most cancers increases dramatically as we age. More than 50% of patients with lung cancer, one of the deadliest cancers worldwide, are diagnosed after the age of 65, and 30% are older than age 70. As the population grows older, the number of lung cancer patients will inevitably increase, leading to a corresponding rise in mortality rates.
Despite the clear connection between aging and cancer, lung cancer is rarely studied in a biological context where the age factor is considered.
The aim of the study is to investigate how aging impacts lung cancer progression and response to anti-cancer therapy lung cancer. Using *in vivo* aged murine models of lung cancer, we observed that age decreased primary tumor burden and increased metastasis incidence. Primary cultures established from primary lung tumors of young and aged mice were used to further study the role of aging in lung cancer progression. Transcriptomics, proteomics, and metabolomics approaches identified strong enrichments of several metabolomic, cytokine and migration pathways in aged primary cultures compared to young primary cultures, leading to the identification of several metabolic vulnerabilities observed in aged cells. We plan to further explore these findings *in vitro* and *in vivo*.
Overall, our results highlight the importance of studying the effect of aging on lung cancer progression, a topic currently overlooked in modern lung cancer research, clinical trials, and treatment options.

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Development and characterization of new mouse models for metastatic bladder cancer

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Metastatic bladder cancer is considered an incurable disease requiring improvement of therapeutic approaches. However, our knowledge of the metastatic spreading process in this disease is still limited and there are very few models that faithfully reproduce it.
We have previously reported that the ablation of two (Pten;Trp53) or four tumor suppressor genes (Rb1;Trp53;Rbl1;Pten) in urothelial cells of the mouse is sufficient to generate aggressive tumors that reproduce specific molecular subtypes of the human disease and also display metastasis dissemination to the lymph nodes, lung, liver or peritoneum. Of note, this property appears to be dependent on the cell of origin (basal or suprabasal urothelial cell) rather than the specific combination of tumor suppressor gene inactivated.
To further study bladder cancer metastasis, we have obtained several primary cell lines from these mouse tumors and used in *in vivo* assays in syngeneic immunocompetent animals. These cell lines also reproduce the molecular portraits of primary tumors and preserve their original organ tropism in metastatic spreading. Furthermore, the metastatic capacities of these cells are increased and accelerated when they are rederived form metastatic lesions. Of note, tumorderived cells showing poor or null metastatic behavior displayed metastatic dissemination with full penetrance in immunodeficient mice, suggesting an immune response responsible to stop metastatic spread in syngeneic mice.
We report the preliminary molecular characterization of these cell lines focusing on different crucial pathways of metastatic processes, such as epithelial mesenchymal transition. We observed that metastasis-rederived cells display increased expression of Twist1, Zeb1 and Prrx1a/b.
In summary, we report the development of unprecedented model system to study the metastasis process in bladder cancer with potential application for the future improvement of clinical management of this deadly disease.

Awarded

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Dissecting brain metastasis response to immunotherapy in new immunocompetent mouse models

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As the therapeutic responses of extracranial tumors continue to improve, brain metastasis (BrM) is increasingly a leading cause of cancer patient mortality. Recent trials of immune checkpoint blockade (ICB) achieved similar response rates to extracranial disease for melanoma patients with asymptomatic BrM. However, the benefit for symptomatic disease is not clear and the mechanisms of resistance remain unknown. The challenge of sample collection from BrM patients limits the identification of key molecular drivers and predictive biomarkers, highlighting the importance of preclinical models. Here we characterize a set of brain metastatic models, developed by intracardiac injection of UV-induced mouse melanoma cell lines, which exhibit diverse histopathology and metastatic potential. Notably, M4- BR1 and M4-BR3 responded differently to ICB mono- and combination therapies at the brain but similarly when implanted subcutaneously in mice. M4-BR1 was highly sensitive to ICB whereas M4-BR3 was resistant at the brain but surprisingly responded at other organs (e.g., liver and bone), mimicking the diversity observed in patients. Single-cell RNA sequencing and high-parametric spectral flow cytometry of untreated M4-BR1 and M4-BR3 metastases revealed marked differences between the models. More than 50% of microglia from M4-BR1 bearing brains presented a distinct phenotype that was associated with increased infiltration of dendritic cells, natural-killer, and a high diversity of T cell subsets, including CD8+, effector T cells. In contrast, M4-BR3 metastases were enriched in neutrophils that are involved in ICB resistance of extracranial melanomas. Mutational and transcriptomic profiling of the brain metastatic melanoma cells uncovered pathways recently identified in human data sets. Our unique mouse cell lines provide a powerful tool for the mechanistic study of BrM progression and immunotherapy response, addressing a critical deficiency in the field.

POSTER SESSION

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Elucidating the role of Aurora Kinase A in Breast cancer Brain Metastasis

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Breast cancer brain metastases (BCBM) represent an aggressive metastatic spread with limited treatment options, partly due to inadequate understanding of its mechanisms. Given that 10-38% of metastatic cases develop BCBM, there is an urgent need for enhanced therapeutic strategies to address this substantial patient population [1]. Leveraging a cohort of patient-matched primary breast and brain metastasis samples, we analysed whole exome sequencing and RNA-seq data for potential vulnerabilities. We identified AURKA as significantly altered in BCBM cases, with 15% amplifications and 56% copy gains across all subtypes (N=38).
RNA-seq data showed elevated AURKA mRNA in BCBM samples (N=63), correlating with adverse survival outcomes. High-throughput drug screen in five BCBM cell lines demonstrated sensitivity to AURKA pathway inhibitors. This finding was corroborated by significant anti-tumour activity upon treatment with AURKA inhibitor, Alisertib in cell lines, patient-derived organoids, and *ex vivo* organotypic cultures. Similar results were observed with siRNA-mediated AURKA knockdowns. Further, transcriptomic changes following AURKA genetic inhibition highlighted differentially expressed genes (DEGs) impacting signalling pathways including MET/VEGF and Notch signalling which were found to be co-vulnerabilities. Particularly, MAML1, a crucial Notch signalling co-activator previously linked to poor prognoses in colon and lung cancers, emerged as a focal point [2]. In our BCBM RNA-seq cohort, high mRNA levels of both AURKA and MAML1 correlated with significantly poorer survival outcomes (N=45). Here, we demonstrate aberrant AURKA expression in BCBM, which is associated with poor prognosis. We propose AURKA as a vulnerable target in BCBM and our current investigation of the kinase-independent downstream effects of AURKA signalling in a BCBM-specific context will likely aid in uncovering the mechanisms driving BCBM progression.

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Cyclin D1 controls the fate of disseminated tumor cells during metastasis

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During metastasis, cells must first evade the primary tumor to reach the blood vessels and enter the circulatory system. Once in the bloodstream, the circulating tumor cells pass through the vessels (extravasation) and colonize new tissues as disseminated tumor cells (DTCs). These DTCs have to survive and establish themselves in the new location to proliferate and form a nodule. Cyclin D1 (Ccnd1) is the regulatory subunit of cyclin-dependent kinase 4 (Cdk4) and has been associated with metastasis in clinical studies. The most widely held view at present is that Ccnd1-Cdk4 would be required for two steps of metastasis: escape of primary tumor cells and proliferation of nodules. Thus, Ccnd1-Cdk4 promotes cell migration and invasion through phosphorylation and activation of Paxillin (Pxn) and triggers cell proliferation through phosphorylation and inactivation of the transcriptional repressor RB1 (retinoblastoma protein).
We have set up a metastasis mouse model by inoculating R3327-5'A cells expressing GFP into the bloodstream of SCID-Hairless mice. At short times after inoculation, we can evaluate the levels of DTCs by counting green cells or clumps in the lungs. At longer times, we can estimate the survival and proliferation of DTCs by counting the number and size of the nodules. Our results suggest that the main role of Ccnd1-Cdk4 in metastasis would be the stimulation of survival and establishment of DTCs. Moreover, we have used genetic approaches, CRISPR technology, and RNA interference to determine the Ccnd1-dependent mechanisms controlling distant organ colonization. We have observed that stimulation of survival of DTCs by Ccnd1 would be independent of RB1 and mediated by the direct or indirect (Pxn-dependent) activation of AKT signaling.

POSTER SESSION

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Deciphering the metabolic dependencies of metastatic melanoma.

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Centro de Biologia Molecular Severo Ochoa, Universidad Autonoma de Madrid

Metastasis formation is the major cause of death in most patients with cancer. In melanoma, the most lethal form of skin cancer (over 70% of skin cancer–related deaths), metastasis to several organs is particularly common and a lethal complication. Yet, the molecular mechanisms and metabolic requirements that cancer cells use to successfully metastasize remain largely undefined. Hence, our goal is to unravel the mitochondrial and metabolic factors that underlie the metastatic potential of melanoma cells.
To identify metabolic and mitochondrial determinates influencing melanoma metastasis, we conducted mitochondrial proteomic analysis on SK-Mel-147 melanoma cells in various contexts; cultured cells, primary tumors, and lung metastases. We found that aldehyde dehydrogenase 2 (ALDH2) was prominently increased in lung metastasis when compared to both primary tumor and culture cells. Subsequently, we confirmed the implication of ALDH2 in promoting melanoma lung metastases by generating Mel-147 ALDH2 knock-out cells which showed a marked decrease in their metastatic capacity.
ALDH2 serves as the primary mitochondrial enzyme responsible for detoxifying reactive aldehydes and mitigating lipid peroxidation within cells. Hence, ALDH2 plays a critical role in detoxification of formaldehyde, acetaldehyde, and other metabolites. On the other hand, ALDH2 activity generates acetate and formate, metabolites that have been shown to be important for the metastatic capacity of cancer cells.
We are presently exploring the mechanisms by which ALDH2 contributes to the formation of metastases. What are the metabolites crucial for the metastatic potential of melanoma cancer cells? Does ALDH2 activity impact the proliferation of melanoma cells once they colonize the lung?

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The contribution of HVEM expression in a leukemia B cell line to the anti-tumor immune response and metastatic dissemination.

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Introduction: A high frequency of mutations affecting the gene encoding Herpes Virus Entry Mediator (HVEM, TNFRSF14) is a common clinical finding in a wide variety of human tumors. Methods: We have addressed how HVEM expression on A20 leukemia cells influences tumor survival, dissemination and assesses its involvement in the modulation of the anti-tumor immune responses in a parental into F1 mouse tumor model of hybrid resistance by knocking-out HVEM expression. HVEM WT or HVEM KO leukemia cells were injected intravenously into semiallogeneic F1 recipients and the extent of tumor dissemination and liver metastases was evaluated in liver and lymphoid organs. Results: The loss of HVEM expression on A20 leukemia eGFP transduced cells led to a significant increase of lymphoid and myeloid tumor cell infiltration in the metastatic nodules of the liver curbing tumor progression. NK cells and to a lesser extent NKT cells and monocytes were the predominant innate populations contributing to the global increase of immune infiltrates in HVEM KO tumors compared to that present in HVEM KO tumors. In the overall increase of the adaptive T cells (both CD4 and CD8 T cells) infiltrating liver metastatic nodules, the stem cell-like PD-1- /Tim 3- T cell progenitors were significantly more abundant than the exhausted and terminally exhausted effector T cell populations (PD-1+ / Tim 3- and PD-1+ / Tim3+), respectively. A significant enhanced immune infiltration of short-lived effector cells (SLECs) and memory precursors of effector T cells (MPECs) in HVEM deficient tumors compared to tumors arisen from HVEM WT tumor cells was also found. Downregulation of Ly108 (CD352, Slamf6) and TCF-1 transcription factor expression occurs as T cells progressed in the course of T cell differentiation towards terminally exhausted T cells concurrently with accumulation of co-inhibitory receptors. Conclusions: The loss of HVEM expression in tumor cells is a disadvantage for the tumor cell survival, dissemination and the formation of liver metastasis. HVEM behaves predominantly as a co-inhibitory receptor in tumor cells since its genetic inactivation enhances immune cell infiltration and improves tumor control. Loss of function mutations in HVEM gene, apart from promoting tumor development, also worsen tumor fitness by unleashing the anti-tumor response. Overall, these findings highlight the role of co-inhibitory signals delivered by HVEM upon engagement of BTLA on T cells and NK cells, placing HVEM/BTLA interaction in the spotlight as a novel immune checkpoint in malignancies of hematopoietic origin.

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A Systems Biology Approach in Unveiling Cancer Metastasis Drug Discovery & Retrospective Clinical Trial Validation

Debabani Roy Chowdhury[1], Sundarajan Kannan[1], Chinmaya Amarkanth[1], Saif Wahid[1], Judhajit Sanyal[1], Tanvi Singh[1*], Ankesh Khemani[1*], Juhi Tayal[2], Anurag Mehta[2*], DC Doval[2*], John Ellingboe[3], Ajith Kamath[1*] and **Arnab Roy Chowdhury[1]**

1. Mestastop Solutions Pvt Ltd, Bangalore India;
2. Rajiv Gandhi Ca

Though ninety percent of cancer deaths are due to metastasis, most current treatments delay the proliferation of the tumour cells in the primary or secondary site, but not the process of metastasis. Attempts to delay metastasis met with failures in the clinic and, therefore, deprioritised. We have adopted a systems biology approach to identify the rate-limiting steps of metastasis by studying the functional biology of isolated and purified primary tumour cells. We replicated metastasis biology on the bench by dissecting and recreating it in thirty functional assays and characterisation steps, which helped differentiate between the properties of moving and growing cells. Patient primary tumour cells, isolated and purified, were further tested on these assay platforms. Wet lab data obtained from these experiments were used as an input into machine learning algorithms. Patient survival, followed over two years, was used as an output to identify the statistically significant steps in defining successful metastasis. Upon identification of critical steps, including invasion, survival in blood and colonisation, we identified four targets that played a crucial role in defining successful metastasis. Genetic engineering of one such target and pharmacological studies using cell lines and primary tumour cells highlighted the platform's utility. We then engineered an orthotopic spontaneous colorectal cancer liver metastasis model to minimise iterative discovery cycle turnaround time, which worked in only six weeks. Together, the two platforms create a robust and specific screening cascade to identify novel first-in-class targets for metastasis drug discovery. Currently, platforms are available for colorectal, head and neck and triple-negative breast cancers. Additionally, to test the clinical relevance of these platforms, we conducted two retrospective clinical trials. Colorectal or head and neck cancer patients with primary tumours and no metastasis were identified, with a follow-up of a minimum of five years. All clinical annotations and medications taken for chronic non-cancer indications were used as input and correlated with survival or death as output. This helped identify non-cancer medications taken chronically that might have any impact on survival or death, with statistical significance. We then tested five such compounds on the *in vitro* platform, and the rank-ordering obtained in inhibiting critical steps of metastasis matched the rank ordering in the retrospective study for promoting survival. *In vivo* studies using a positive compound showed a statistically significant reduction of liver metastasis without impacting the primary tumour size. Conversely, a weaker compound did not affect proliferation or metastasis. Together, our wet lab-generated *in vitro* and *in vivo* platforms synergise with machine learning algorithms to help unveil novel targets for cancer metastasis and will be instrumental for drug discovery and drug repurposing to identify novel candidates for metastasis treatment in an adjuvant setting.

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Multiomics in patient-derived breast cancer organoids establish IL6/STAT3 signalling as a driver of metastatic progression

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Malignant fluid accumulation in the pleura or the peritoneum of breast cancer (BCa) patients is common in advanced metastatic disease. Such metastatic fluids are commonly drained as palliative care for patients. Here we aim to understand what regulates metastatic BCa cells in ascites (ASC) and pleural effusions (PE) to discover novel therapeutic targets.

We prospectively reviewed clinical records of 133 advanced BCa patients (64.7% ASC vs. 35.3% PE). Although ASC-harbouring patients built-up fluid later after metastatic relapse (average time ASC=797 vs. PE=462 days, p-value<0.005), those with PE survived for longer after the first drainage (mean survival time ASC=56.5 vs. PE=128 days, p-value<0.05).

We profiled metastatic BCa cells from 6 patients using multiomics. Integration of transcriptomics, phospho- and proteomics revealed that metastatic BCa cells are biologically different according to fluid type, suggesting these patients should be clinically treated as such. We showed that metastatic fluid itself promoted patientderived organoid (PDO) growth *in vitro*. Next, we investigated BCa metastatic fluids using mass spectrometry and cytokine profiling. Interestingly, we observed high levels of IL6 in these patient-derived malignant fluids (range 4-30 ng/mL). IL6 stimulated growth via STAT3 signalling and increased breast cancer stem cell activity in cell lines, PDOs and patient-derived xenograft organoids which were reversed using the IL6 antagonist, tocilizumab. Furthermore, metastatic fluid containing IL6 promoted BCa growth (2.5-fold) and metastatic burden (3-fold) in an *in vivo* model of metastatic fluid accumulation.

The multiomic approach identified potential therapeutic targets in advanced BCa and showed that ASC and PE are phenotypically different. We established that IL6 regulates BCa progression using PDOs and an ascites *in vivo* model. Thus, repurposing drugs such as tocilizumab will benefit the clinical management of advanced metastatic BCa patients.

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Adoptive NK cell therapy as a tentative treatment for colorectal cancer lung metastasis

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7. Thoracic Surgery Department, Hospital Universitario Miguel Servet and Hospital Clínico Universitario Lozano Blesa.
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We have identified a cluster of metastases from different tumor types that exhibit an inflammatory phenotype. Most of these high immune metastases are lung metastases (Garcia-Mulero et al, JITC, 2020). Also, we have demonstrated that NK cells control CRC development (Lanuza et al. Front Immun, 2022). Thus, we hypothesize that CRC lung metastases are more likely to benefit from immunotherapy. First, a molecular characterization comparing lung and liver metastases was done. Gene expression data of lung metastases (n=35) and liver metastases (n=153) from CRC were downloaded and used to perform an immune characterization of the samples. Mutational data from 624 liver metastases and 146 lung metastases were downloaded from cBioPortal. Lung metastases showed higher immunogenic scores than liver metastases: immunophenoscore, p=2e-10; Antigen presentation score, p=1.7e-10; Cytotoxic T cells, p=1.9e-7; Regulatory T cells, p=9.2e-6; B cells, p=0.001; Dendritic cells, p=1.5e-5; NK cells, p=5.3e-8. Gene set enrichment analysis showed interleukin and interferon pathways enriched in lung metastases. At genomic level, no differences were found in total number of mutations but KRAS and FBXW7 mutations were more frequent in lung metastases. Second, fresh surgical specimens of lung metastasis from CRC patients were obtained after surgical resection and orthotopically implanted. As a proof-ofconcept (PoC), one of these models were used to test NK treatment. Three groups of mice treated with vehicle (n=3), 5-fluorouracil + irinotecan (n=3), and vehicle + NK92 (n=3) were included in the experiment. the PoC study demonstrated the NK cells treatment feasibility in ortoxenografts models and the efficacy in tumor control. All treated animals showed a significant reduction in tumor weight and tumor size in comparison with animals treated with standard chemotherapy. In conclusion, CRC lung metastases have a favourable immunophenotype that makes them a promising target to NK cell treatment.

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Epigenetic characterization of partial-EMT state in Oral Squamous Cell Carcinomas

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Oral squamous cell carcinomas (OSCCs) represent one of the most common head and neck cancers (HNCs), which are mostly caused by tobacco and alcohol exposure. These have high risk of recurrence and metastasis, and low survival rate. Consequently, there is an actual need for understanding the molecular processes underlying OSCC progression. Recent scRNAseq analysis on HNCs discovered a discrete population of cancer cells expressing a signature of partial epithelial to mesenchymal transition (pEMT)1, which was correlated with the presence of metastatic disease. In cutaneous-SCC, which are related to HNCs, pEMT state is defined at the transcriptional level by the co-expression of epithelial (TP63/SOX2) and mesenchymal (SNAI1/2, ZEB1/2) transcription factors (TFs)2. Interestingly, HNCs pEMT positive cells failed to co-express canonical mesenchymal TFs1. To understand how pEMT programs are regulated in OSCCs, we have purified pEMT low and high tumor cells from OSCCs patient biopsies to perform bulk RNAseq and characterized potential signaling pathways and TFs controlling their function. Within the top upregulated genes, we have identified a signature that can classify patients at risk of developing aggressive OSCC with metastatic spread and low survival rates. In parallel, we have profiled their chromatin accessibility and histone modifications to detect TF binding and enhancer activities regulating pEMT cells. Interestingly, our data suggest that pEMT plastic phenotype may be controlled coordinately by EMT and epithelial SCC TF, and some epigenetic remodelers that are specifically expressed in pEMT. Using patient-derived organoid models that preserve genetic and epigenetic pEMT characteristics observed in the primary tumor, we are beginning to unravel the role of specific genes in tumor cell behavior, therapy resistance and metastatic spread.

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Evaluation of doxorubicin on an invasive breast cancer in vitro model

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INTRODUCTION. Breast cancer (BC) is the greatest cause of mortality for women globally, with metastasis serving as the main contributing factor. Despite major advances in the pharmacological treatment of BC, chemotherapy remains the first line of attack, administered both pre- and post-surgery to reduce the tumor size and cancer cell dissemination. Considering the efficacy of chemotherapy is largely dependent on the rapid proliferation rate of cancer cells [1], the question arises as to whether the invasive cells are as affected as the cells from the primary tumor [2]. To delve into this matter, we examined the effects of doxorubicin on MDA-MB-231 invasive breast cancer cells using a 3D-ECM model. RESULTS & DISCUSSION. -The 3D-ECM model demonstrated reduced sensitivity to doxorubicin compared to traditional *in vitro* models. By evaluating the system's viability, we verified that our design effectively mimics the tumor's physiological response to doxorubicin. Notably, spheroids displayed greater viability than cells on 2D surfaces and those dispersed in ECM. This indicates that beyond just the ECM, the spheroidal arrangement simulates better *in vivo* solid tumor resistance to chemotherapeutics. - Once MDA-MB-231 cells invade the matrix, they exhibit increased resistance to doxorubicin. While the invasive capability of the spheroid cells is compromised after doxorubicin treatment, cells that have already invaded remain unaffected, suggesting the emergence of evasion or resistance mechanisms along with the ability to invade.-The invasive behavior of MDA-MB-231 cells is amplified on a microfluidic chip. Tests conducted with spheroids included on microfluidic devices with two channels separated by a porous membrane (Be- Transflow chip, BeonChip) facilitated the creation of a chemoattractant gradient, driving directional cell invasion towards the membrane, which may potentially enable the isolation of a circulating cell (CTCs) phenotype at the perfusable channel underneath.

METHODS. Large spheroids were employed to replicate the core attributes of the primary tumor and a collagen I hydrogel (3 mg/mL) was used to mimic the tumor ECM. Within this model setup, invasive cells can emerge from the spheroid driven by resource availability and progress across the matrix. Doxorubicin was administered following the standard BC treatment scheme at half and twice the concentration extrapolated from levels detected in patients' bloodstreams.

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Understanding the Link Between Platelet Mitochondria Transfer and Prostate Cancer Progression

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Instituto Nacional de Medicina Genómica (INMEGEN)
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BACKGROUND: Prostate cancer (PCa) is the second most common neoplasia in men worldwide. The interaction of cancer cells with cells present in the tumor microenvironment, as platelets, might influence tumor progression. Platelets are cells playing an essential role in homeostasis. Platelets play an important role in protecting cancer cells from the immune response, influencing their growth, and spread. Platelets contain components as granules, RNA, proteins, and mitochondria, which can be exchanged in both directions during interactions with cancer cells. Mitochondria transfer is a physiological process to maintain homeostasis, however, it is also observed in cancer. The transfer process includes nanotube formation, cell fusion, gap junctions, and exchange of extracellular microvesicles (MVs). This mitochondria transfer leads to a metabolic rewriting in the recipient cells. Recently, it has been described that activated platelets might release mitochondria-containing MVs. In addition, it has shown platelets can be activated by PCa cells. The role of the platelet mitochondria transfer in PCa has not been explored. The aim of the study is to determine the mitochondrial transfer from platelets to PCa cells and describe the metabolic effects on PCa cells and the relation with tumor progression.
METHODS: Platelets were isolated from healthy volunteers and co-cultured with PCa cell lines. PCa cell lines were exposed to platelets, and mitochondrial transfer was analyzed through MitoTracker dyes using fluorescence assays and flow cytometry. Additionally, the effect of platelets on NADH, lactate and ATP production in PCa cell lines was analyzed by fluorescence assays.
RESULTS: Platelets exposed to PCa cell lines release mitochondria in MVs and release free mitochondria. These mitochondria were internalized by the PCa cell lines. In addition, metabolism assays showed platelet mitochondria transfer alters the levels of NADH, lactate and ATP in PCa cell lines.
CONCLUSIONS: Activated platelets are capable of transferring mitochondria to PCa cell lines. This transfer promotes changes in the metabolism of PCa cell lines that might support tumor progression. Further research is necessary to validate whether platelet mitochondria transfer influences tumor progression in PCa. My apologizes but, I could not add my conclusions in the abstract section. In the information of the abstract content, it says that maximum words are 450 but the abstract section says that only 2000 chacarcterers including spaces. So, I included in this section my conclusions.

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57 Lack of RIPK4 reduced Wnt3A-stimulated migration and invasion potential in melanoma cells

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Melanoma, which is one of the most aggressive and deadly skin cancers, may be resistant to conventional therapies, which is why new mechanisms are being sought for its treatment. Receptor interacting protein kinase 4 (RIPK4) may be a potential target.

RIPK4 is a serine/threonine kinase that targets multiple signaling pathways including Wnt/β-catenin and plays an important role in the regulation of cell proliferation, invasion and metastasis in several malignancies. However, the role of RIPK4 on the regulation of the Wnt/β-catenin pathway in melanoma is still largely unknown.

In this study, we determined the effects of genetic manipulation of RIPK4 on Wnt3A – stimulated invasiveness in human malignant melanoma: A375 and WM266.4 cells. Downregulation of RIPK4 was performed in A375 and WM266.4 melanoma cell lines using CRISPR/Cas9 technique, and RIPK4 levels were verified by Western blot. We observed that RIPK4 knockout impaired Wnt3A-induced activation of Wnt/β-catenin pathway manifested by a decrease in the transcriptional activity of β-catenin in Top-Flash reporter system in both tested melanoma cell lines, A375 and WM266.4. Importantly, Western Blot analysis showed that the expression of proteins related with invasion as N-cadherin, Vimentin and MMP9 was decreased in both A375 and WM266.4 upon RIPK4 knockdown, suggesting that this kinase may act on pathways that consequently affect the metastatic capacity of melanoma cells. Moreover, RIPK4 knockout led to the inhibition of scratch overgrowth, migration and invasion of these cells compared to their controls.

In conclusion, RIPK4 kinase directly affects signal transduction through the canonical Wnt/β-catenin pathway in melanoma and plays an important role melanoma cells invasion. The results of the presented study

Acknowledgements
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Madrid 6th – 8th November 2023

Metastasis

Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

2023

DIET, NUTRITION AND CANCER CELL METABOLISM
22/05/2023 – 23/05/2023
Organisers: Felipe Cortés, Óscar Fernández-Capetillo, Ana Losada, Andre Nussenzweig

2022

DIET, NUTRITION AND CANCER CELL METABOLISM
24/10/2022 – 26/10/2022
Organisers: Nabil Djouder, Nikla Emambokus, M. Carmen Fernández-Agüera, Valter Longo, Marcos Malumbres

MOLECULAR, CELLULAR AND ORGANISMAL DRIVERS OF AGING
09/05/2022 – 10/05/2022
Organisers: Maria A. Blasco, Alejo Efeyan, Thomas Rando

2019

HETEROGENEITY AND EVOLUTION IN CANCER
23/09/2019 – 25/09/2019
Organisers: Fátima Al-Shahrour, Arnold Levine, Solip Park, Raúl Rabadán

ESTRUCTURAL AND MOLECULAR BIOLOGY OF THE DNA DAMAGE RESPONSE
20/05/2019 – 22/05/2019
Organisers: Oscar Llorca, Rafael Fernández Leiro, Laurence H. Pearl, Titia Sixma

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

2018

MOLECULAR, CELLULAR AND ORGANISMAL HALLMARKS OF AGING
07/05/2018 – 09/05/2018
Organisers: Maria A. Blasco, Alejo Efeyan, Kathleen Collins, Thomas Rando

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY
09/07/2018 – 11/07/2018
Organisers: Victoria Aranda, Nabil Djouder, Joao Monteiro, Marisol Soengas, Laurence Zitvogel

2017

PRIMARY AND SECONDARY BRAIN TUMORS
19/02/2017 – 22/02/2017
Organisers: Massimo Squatrito, Manuel Valiente, Richard Gilbertson, Michael Weller

MOLECULAR CHAPERONES IN CANCER
02/05/2017 – 04/05/2017
Organisers: Nabil Djouder, Wilhelm Krek, Paul Workman, Xiaohong Helena Yang

2016

CANCEROMATICS III – TUMOR HETEROGENEITY
13/11/2016 – 16/11/2016
Organisers: Fátima Al-Shahrour, Núria Malats, Alfonso Valencia, Chris Sander

Metastasis

2015

METASTASIS INITIATION:
MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES
28/09/2015 - 30/09/2015
Organisers: David Lyden, Yibin Kang, Gemma Alderton, Victoria Aranda, Li-kuo Su, Héctor Peinado

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT
22/03/2015 - 25/03/2015
Organisers: Manuel Hidalgo, Alberto Bardelli, Lillian Siu, Josep Tabernero

2013

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER
27/05/2013 - 29/05/2013
Organisers: Robert Benezra, Ana Losada, Marcos Malumbres, René Medema

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING
17/09/2012 - 19/09/2012
Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

2011

RECAPTURING PLURIPOTENCY:
LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER
07/11/2011 - 09/11/2011
Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

CANCEROMATICS II :
MULTILEVEL INTERPRETATION OF CANCER GENOME
28/03/2011 - 30/03/2011
Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER
07/02/2011 - 09/02/2011
Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS:
PERSONALIZING MEDICINE
22/11/2010 - 24/11/2010
Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS
08/03/2010 - 10/03/2010
Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

Metastasis

2009

THE ENERGY OF CANCER
02/11/2009 - 04/11/2009
Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

CANCER-OM-ATICS II: MULTILEVEL INTERPRETATION OF CANCER GENOME
06/07/2009 - 08/07/2009
Organisers**:** Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER
23/02/2009 - 25/02/2009
Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

2008

SIGNALLING UPSTREAM OF mTOR
03/11/2008 - 05/11/2008
Organisers: Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sánchez-Céspedes

STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL
23/06/2008 - 25/06/2008
Organisers: Niko Grigorieff, Eva Nogales and Jose María Valpuesta

DEVELOPMENT AND CANCER
04/02/2008 - 06/02/2008
Organisers: Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY
05/11/2007 - 07/11/2007
Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS
11/06/2007 - 13/06/2007
Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM
19/02/2007 - 21/02/2007
Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSEF STEINER CANCER CONFERENCE
13/11/2006 - 15/11/2006
Organisers: Maria A. Blasco and Jerry Shay

MEDICINAL CHEMISTRY IN ONCOLOGY
02/10/2006 - 04/10/2006
Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER
22/05/2006 - 24/05/2006
Organisers: Curtis Harris, Raymand Dubois, Jorge Moscat and Manuel Serrano

PTEN AND THE AKT ROUTE
08/05/2006 - 10/05/2006
Organisers: Ana Carrera, Pier Paolo Pandolfi and Peter Vogt

2005

CANCER AND AGING
07/11/2005 - 09/11/2005
Organisers: Maria A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano

MAP KINASES AND CANCER
30/05/2005 - 01/06/2005
Organisers: Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda

ANIMAL TUMOUR MODELS AND FUNCTIONAL GENOMICS
07/03/2005 - 09/03/2005
Organisers: Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks

2004

CADHERINS, CATENINS AND CANCER
29/11/2004 - 01/12/2004
Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS
27/09/2004 - 29/09/2004
Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

CNIO FRONTIERS MEETINGS and CNIO CANCER CONFERENCES

2003

APOPTOSIS AND CANCER
01/12/2003 - 03/12/2003
Organisers: Gabriel Nuñez, Marisol Soengas and Scott Lowe

SMALL GTPases IN HUMAN CARCINOGENESIS
16/06/2003 - 18/06/2003
Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

TARGETED SEARCH FOR ANTICANCER DRUGS
17/03/2003 - 19/03/2003
Organisers: Amancio Carnero and David H. Beach

2002

MECHANISMS OF INVASION AND METASTASIS
18/11/2002 - 20/11/2002
Organisers: Maria A. Blasco and Jerry Shay

THE CELL CYCLE AND CANCER
30/09/2002 - 02/10/2002
Organisers: Marcos Malumbres, Charles Sherr and Jiri Bartek

CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN
29/05/2002 - 31/05/2002
Organisers: Manel Esteller and Stephen B. Baylin

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cnio – CaixaResearch FRONTIERS MEETINGS

June 10th – 12th , 2024
Venue: CNIO Auditorium — Madrid • Spain
Abstract submission deadline: May 27, 2024
Registration deadline: May 6, 2024

Molecular chaperones in cancer and protein quality control

Organising committee

Nabil Djouder
Spanish National Cancer Research Centre-CNIO, Spain

Gabriela Chiosis
Memorial Sloan Kettering Institute, US

Judith Frydman
Stanford University, US

Óscar Llorca
Spanish National Cancer Research Centre-CNIO, Spain

Paul Workman
The Institute of Cancer Research, London, UK

Speakers

Udai Banerji
The Institute of Cancer Research; The Royal Marsden NHS Foundation Trust, UK

Jeffrey L. Brodsky
University of Pittsburgh, Kenneth P. Dietrich School of Arts and Sciences, US

Johannes Buchner
Technical University of Munich, Germany

Benoit Coulombe
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Max-Planck-Institute of Biochemistry, Germany

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University of Michigan, US

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Center for Molecular Biology Heidelberg – ZMBH, Germany

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Center for Cancer Research, National Cancer Institute, US

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University of Leeds, UK

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Babraham Institute, US

Lea Sistonen
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Peter Walter
Howard Hughes Medical Institute, University of California, US

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FORTHCOMING CNIO-CAIXARESEARCH FRONTIERS MEETINGS

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cnio – CaixaResearch FRONTIERS MEETINGS

October 16th – 18th , 2024
Venue: CNIO Auditorium — Madrid • Spain
Abstract submission deadline: September 16, 2024
Registration deadline: October 2, 2024

Frontiers in immunomodulation and cancer therapy: 2nd edition

Organising committee

Luis Álvarez-Vallina
Spanish National Cancer Research Centre – CNIO, Spain

María Casanova-Acebes
Spanish National Cancer Research Centre – CNIO, Spain

Nabil Djouder
Spanish National Cancer Research Centre – CNIO, Spain

David Sancho
Spanish National Centre for Cardiovascular Research – CNIC, Spain

Xin (Cindy) Sun
JEM - Rockefeller University Press, US

Speakers

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NIH/National Institute of Allergy and Infectious Diseases, US

Sandra Demaria
Weill Cornell Medical College, US

Florent Ginhoux
Gustave Roussy, France

Mark Cragg
Southampton General Hospital-University of Southampton, UK

Kenneth Murphy
Washington University in St. Louis, US

Karolina Palucka
The JAX Cancer Center, US

Boris Reizis
NYU Grossman School of Medicine, US

Bruno Silva-Santos
Institute of Molecular Medicine (IMM) João Lobo Antunes, Portugal

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Institute for Immunology, Tsinghua University, China

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Madrid 6th – 8th November 2023

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MEETINGS

Madrid 6th – 8th November 2023

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As a non-profit organisation, we would like to thank all those who sponsored this conference.
Such contribution helps us to ensure that our conferences will continue to establish the CNIO
as a point of reference for the international cancer research community.

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