7TH ASPIC

INTERNATIONAL CONGRESS

25-26 JUNE, 2026

IPO-PORTO • PORTUGAL

PROCEEDINGS BOOK

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LETTER OF WELCOME

Dear Colleagues and Friends,

As Presidents of the 7th ASPIC International Congress, and on behalf of the ASPIC Board, as well as the Organizing and Scientific Committees, it is with great enthusiasm that we invite you to attend this upcoming edition, taking place on June 25th and 26th, 2026, at the IPO Porto Auditorium in Porto, Portugal.

After the great success of the 2024 Congress held in the Algarve, we are excited to bring the ASPIC Congress back to Porto. This seventh edition promises to be another rewarding and outstanding scientific meeting. Our goal is to highlight the cutting-edge cancer research being conducted in Portugal, while also welcoming leading international experts to ensure the highest scientific standards.

We warmly encourage you to take an active part in the Congress. It will be an excellent opportunity to engage with a vibrant scientific programme and to establish new contacts and collaborations. The meeting will feature oral presentations and two dynamic and participative Poster Sessions, providing a platform for sharing results, discoveries, and ideas.

ASPIC also remains committed to strengthening the dialogue between researchers, oncologists, and patient advocates. In this spirit, we are pleased to announce that we will once again organize the ASPIC Patients' Advocacy Poster Walkthrough 2026 to promote the exchange of perspectives and knowledge. Our aim is to foster new research directions and scientific discoveries that may have a meaningful impact on the lives and treatment of cancer patients.

We look forward to welcoming you to Porto for what we are confident will be an inspiring and engaging event.

Júlio Oliveira and **Vítor Veloso**

(Presidents of the 7th ASPIC International Congress)

TABLE OF CONTENTS

CONGRESS COMMITTEES

Scientific & Organizing Committee

Carmen Jerónimo

Joana Paredes

João Taborda Barata

Luísa Melo

Rui M. Reis

Sandra Casimiro

Scientific Evaluation Committee

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Ana Sofia Ribeiro

Ana-Teresa Maia

Branca Cavaco

Celso Reis

João Lobo

Marta Soares

Rita Fior

CONGRESS PROGRAMME

THURSDAY, 25TH JUNE

09:00 • Official Opening

Carmen Jerónimo (ASPIC, CI-IPOPorto/ICBAS-UP, PT), **Júlio Oliveira** (IPOPorto) and **Vítor Veloso** (LPCC-NRN)

OPENING SESSIONS

Chair: **João Barata** and **Sandra Casimiro**

09:30 • EACR Lecture: From Screens to Solutions: CRISPR-Guided Target Discovery and Drug Combination Design

Roderick L. Beijersbergen (NKI, The Netherlands)

10:00 • EAU Section of Urological Research Lecture: Using liquid biopsy to personalize treatment and improve outcomes in bladder cancer

Lars Dyrskjøt (Aarhus University, DK)

10:30 • ASEICA Lecture: Liquid biopsy in breast cancer

Rafael López (President of ASEICA, IDIS, Spain)

11:00 • Coffee Break

SYMPOSIUM I – ENVIRONMENTAL AND HEREDITARY RISK FACTORS

Chair: **Ana-Teresa Maia** and **Rui Reis**

11:30 • Cancer Syndromes: Genotype–phenotype associations and the cost of complex care pathways

Carla Oliveira (i3S, Porto, Portugal)

12:00 • SELECTED ORAL COMMUNICATION

Systemic Reprogramming of Hematopoiesis by Colorectal Tumors

Ana Luísa Machado (i3S, Porto, Portugal)

12:15 • Environmental risk factors for early onset colorectal cancer

Sandra Perdomo (Genetic Section of IARC, Lyon, France)

12:45 • SELECTED ORAL COMMUNICATION

ADARB1 drives nuclear plasticity downstream of E-cadherin loss in Hereditary Diffuse Gastric Cancer

Luísa Carvalho (i3S, Porto, Portugal)

13:00 • Lunch

14:00 • Poster Session (odd numbers) and Patients' Advocacy Poster Walkthrough

16:00 • Coffee Break

SYMPOSIUM II – EMERGENT HALLMARKS IN CANCER

Chairs: **João Lobo** and **Céu Figueiredo**

16:30 • Sticky situation: Bacterial-epithelial contact drives colorectal cancer and IgA pathology

Lars Vereecke (CRIG e VIB, Belgium)

17:00 • SELECTED ORAL COMMUNICATION

Rab27a Loss Defines an Inflammatory and Therapeutically Vulnerable Subtype of Pancreatic Cancer

Carolina Dias (i3S, Porto, Portugal)

17:15 • Phenotypic plasticity

Adriana Sánchez-Danés (Fundação Champalimaud, Lisbon, Portugal)

17:45 • SELECTED ORAL COMMUNICATION

Macrophage-driven survival pathways enable resistance to radiotherapy in triple-negative breast cancer

Ana Manuela Borges (i3S, Porto, Portugal)

18:00 • ASPIC General Assembly and Elections

20:00 • Congress Dinner

FRIDAY, 26TH JUNE

09:00 • USA-PT Initiative against Cancer (UPIC) Lecture: Sensory neurons are needed for both initiation and progression of stomach cancer

Chair: **Joana Paredes** and **Rita Fior**

Timothy C. Wang (Columbia University, USA)

ASPIC-SPO JOINT SYMPOSIUM

Chairs: **Marta Soares** (ASPIC) and **Nuno Bonito** (SPO)

09:30 • Liquid biopsies as a prognostic factor – Colorectal cancer as a model

Nuno Bonito (Pr. SPO, IPO-Coimbra, Portugal)

10:00 • Precision medicine in the treatment of lung cancer

João Moreira Pinto (OncMed HLuz, Lisboa, Portugal)

10:30 • RLTs in earlier disease setting: current status and future perspectives

James Nagarajah (Radboud University Medical Center, The Netherlands)

11:00 • Coffee Break

SYMPOSIUM III – CANCER CELL MECHANISMS

Chairs: **Celso Reis** and **Célia Gomes**

11:30 • Mechano-metabolic adaptations during metastatic dissemination

Vittoria Graziani (ICR, AC, UK)

12:00 • SELECTED ORAL COMMUNICATION

Multi-matrix proteomics reveal urine-derived extracellular vesicles as an enriched source of bladder cancer-specific proteins

Catarina Lourenço (CI-IPO Porto, Portugal)

12:15 • Lysosome exocytosis as a therapeutic target in cancer progression

Duarte Barral (NOVA Medical School, Lisbon, Portugal)

12:45 • SELECTED ORAL COMMUNICATION

Development of a microRNA-regulated cell death-inducing gene therapy for T-cell Acute Lymphoblastic Leukemia

Mafalda Duque (GIMM, Lisboa, Portugal)

13:00 • Lunch

14:00 • Poster Session (even numbers) and Patients' Advocacy Poster Walkthrough

16:00 • Coffee Break

ASPIC – PORTO.CCC RAQUEL SERUCA JOINT SYMPOSIUM – CLINICAL TRIALS DRIVEN BY BIOMARKERS

Chairs: **Carmen Jerónimo** and **José Carlos Machado**

16:30 • Genomic guided platform studies Basket of Baskets

Irene Braña (Vall d'Hebron Institute of Oncology, Spain)

17:00 • SELECTED ORAL COMMUNICATION

Novel CAR T formulations targeting tumor-associated glycoepitopes: A new strategy for solid tumorser

Rafaela Abrantes (i3S, Porto, Portugal)

17:15 • Delivering precision immunotherapy with MANIFEST and RISE

Jonathan Lim (The Christie NHS Foundation Trust, UK)

17:45 • SELECTED ORAL COMMUNICATION

Fishing for new immunotherapy compounds to boost innate-tumor rejection

Rita Fior (F.Champalimaud, Lisbon, Portugal)

18:00 • Towards academic Point-of-Care CAR-T cell therapy in the Netherlands

Edwin Bremer (University Medical Center Groningen, The Netherlands)

18:30 • Awards & Closing Session

ABSTRACTS

From Screens to Solutions: CRISPR-Guided Target Discovery and Drug Combination Design

Author and Affiliation

Roderick L. Beijersbergen

NKI, The Netherlands

Abstract

Identifying robust therapeutic targets and effective drug combinations in heterogeneous, clinically relevant models remains a major barrier to translating genomic discoveries into patient benefit. To address this, we deployed large-scale CRISPR loss- and gain-of-function screens across multiple in vitro, in vivo, and patient-derived models to systematically nominate genetic dependencies, then integrated these results with focused combinatorial drug testing and high-resolution phenotyping to prioritize clinically actionable strategies. Screens across models revealed both conserved and context-specific vulnerabilities, enabling rigorous prioritization of targets with translational potential. Subsequent combinatorial testing uncovered novel synergistic drug pairs that enhanced efficacy and mitigated resistance in model systems, and insight into cell-type-specific responses and microenvironmental influences on sensitivity. This integrated pipeline bridges target discovery and therapeutic development by identifying target–combination pairs with strong preclinical evidence and mechanistic rationale for clinical translation.

Using liquid biopsy to personalize treatment and improve outcomes in bladder cancer

Author and Affiliation

Lars Dyrskjøt

Aarhus University, DK

Abstract

Muscle-invasive bladder cancer (MIBC) is entering an increasingly complex treatment landscape, where perioperative systemic therapy, radical cystectomy and organ-preserving approaches are being developed in parallel. This creates a need for biomarkers that move beyond prognostic classification and support biologically informed treatment selection. Although the most mature clinical evidence currently comes from post-cystectomy studies, these data provide an important framework for translating molecular residual disease concepts into bladder preservation.

This talk will review tumour-based and liquid biopsy biomarkers in MIBC, including molecular subtypes, immune-related features and DNA damage response alterations, with particular emphasis on circulating tumour DNA (ctDNA) as a marker of molecular residual disease, relapse risk and treatment response. Key evidence from ctDNA-guided perioperative and adjuvant studies, including IMvigor011, TOMBOLA and related prospective work, will be discussed as examples of how serial molecular testing may support treatment escalation and de-escalation.

Building on this framework, the talk will address how biomarker concepts are entering the bladder preservation field. Plasma ctDNA and urine tumour DNA may provide complementary information: plasma analyses can inform systemic molecular residual disease and metastatic risk, whereas urine-based assays may be particularly relevant for local residual disease, intravesical recurrence and response to organ-preserving treatment. Emerging translational data from bladder-sparing trials will be highlighted.

Finally, the presentation will discuss how these approaches can be moved towards clinical implementation, and what evidence is needed before biomarkers can guide decisions about treatment intensification, de-escalation or bladder preservation.

ASEICA Lecture: Liquid biopsy in breast cancer

Authors and Affiliations

Rafael López

President of ASEICA, IDIS, Spain

Abstract

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SYMPOSIUM I
ENVIRONMENTAL AND HEREDITARY RISK FACTORS

Cancer Syndromes: Genotype–phenotype associations and the cost of complex care pathways

Author and Affiliation

Carla Oliveira

i3S, Porto, Portugal

Abstract

SELECTED ORAL COMMUNICATION

Systemic Reprogramming of Hematopoiesis by Colorectal Tumors

Authors and Affiliations

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15. UCIBIO - Unidade de BioCiências Moleculares Aplicadas, Instituto de Saúde e Bioeconomia, Universidade do Porto

* Currently at Blueclinical, Porto, Portugal

Abstract

Introduction: Classically, immune escape is studied locally within the tumor microenvironment. Nonetheless, tumors communicate with the whole body, influencing the host systemically. Herein, we used a genetically-engineered mouse model of intestinal cancer, the Msh2/Kras, to study the systemic effects of tumors on the host organism.

Material and Methods: A genetically engineered mouse model harboring knockout of Msh2 combined with oncogenic Kras activation (Msh2Kras) was employed to recapitulate colorectal carcinogenesis. Hematopoietic organs and tumor tissues were subsequently characterized through flow cytometric immunophenotyping and hematoxylin-eosin histological staining. Serum cytokines concentration was measured through multiplex cytokine array. RNA-Seq was performed on tumor samples to assess transcriptomic profiles.

Results: We observed that oncogenic Kras accelerated tumor growth, with Msh2/Kras mice displaying multiple tumors throughout the intestine at 20 weeks of age. In comparison, Msh2 single mutant animals showed delayed tumor onset, developing fewer intestinal tumors at 40 weeks of age. Spleens of Msh2/Kras and Msh2 tumor-bearing mice revealed alterations in distinct immune cell populations compared with age-matched wild-type and tumor-free single mutant mice. Additionally, the analysis of the bone marrow hematopoietic progenitor cells revealed an expansion of the myeloid progenitors, suggesting a tumor-induced chronic "emergency" granulopoiesis in both Msh2/Kras and Msh2.

To identify potential mediators of tumor-hematopoietic crosstalk, we observed an upregulation of cytokines involved in neutrophil recruitment and activation in the blood serum of tumor-bearing mice. Notably, tumor-bearing mice showed a phenotypic shift toward immature neutrophil differentiation that extends from the bone marrow to the mice blood, and to the tumors in both Msh2 and Msh2Kras. Transcriptomic profiling revealed that the predominant transcriptional differences were the increased activation of inflammation-related pathways in Msh2 single mutants compared to Msh2Kras animals, suggesting a different modulation of the local immune compartment. Currently, to functionally validate the differences observed between Msh2 and Msh2Kras animals we are co-culturing organoids of both models with neutrophils to explore whether the different tumoral context can influence the neutrophils effector functions.

Conclusion: Our study highlights that intestinal tumors regardless of genotype establish a long-distance communication with the bone marrow impairing neutrophils maturation. Though, the specificities of the tumor immune microenvironment are potentially mediated by the tumor genotype. Understanding these processes is crucial for developing new therapeutic strategies aimed at strengthening the immune response against nascent tumors, preventing their escape, and ultimately slowing the progression towards malignancy.

Environmental risk factors for early onset colorectal cancer

Author and Affiliation

Sandra Perdomo

Genetic Section of IARC, Lyon, France

Abstract

Over the past two decades, colorectal cancer (CRC) incidence in individuals younger than 50, referred to as early-onset colorectal cancer (eoCRC), has doubled in many countries. The reasons for this increase are uncertain, especially as CRC rates at later ages are decreasing in most countries. In this talk, I present extensive evidence that has emerged supporting the hypothesis that certain mutagenic bacteria, including E. Coli pks+, in the colorectal microbiome of infants and children across multiple countries has led to an increased mutation load in colorectal epithelial cells early in life, which might be responsible for the rising incidence of eoCRC. Additional studies are examining the interaction between these bacteria-derived mutation profiles and other suspected risk factors responsible for the increase in eoCRC, such as obesity at younger ages and the consumption of ultra-processed foods.

SELECTED ORAL COMMUNICATION

ADARB1 drives nuclear plasticity downstream of E-cadherin loss in Hereditary Diffuse Gastric Cancer

Authors and Affiliations

Luísa Carvalho[1,2,3], Joana Pereira[1,2,3], Soraia Melo[1,2,4], José Pedro Santos[1,2,3], Diogo Fróis Vieira[4], José Carlos Machado[1,2,3], Eurico Morais-de-Sá[1,5], João Miguel Sanches[4], Joana Figueiredo[1,2,3,#]

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Abstract

Hereditary diffuse gastric cancer is an autosomal dominant cancer syndrome caused by germline variants in the CDH1 gene, encoding E-cadherin. E-cadherin dysfunction is strongly associated with the ability of cancer cells to invade and disseminate into the peritoneal cavity and adjacent organs. However, the molecular mechanisms driving this invasive behaviour remain poorly understood. We hypothesise that loss of E-cadherin activates a mechanotransduction programme that promotes nuclear remodelling and a consequent pro-invasive signature.

We established in vitro cell models expressing either wild-type or mutant E-cadherin forms found in HDGC patients. Nuclear proteomic profiling was performed using high-resolution mass spectrometry, which identified the nuclear protein ADARB1 as differentially regulated. To investigate the biological relevance of ADARB1 in this context, we modulated its expression and assessed nuclear architectural features during cell migration both in vitro and in vivo.

Proteomic analysis of nuclear profiles revealed a consistent decrease of ADARB1 in mutant cells. Silencing of ADARB1 in wild-type cells led to an enhanced migratory capacity, characterised by more direct and confined movement with limited interaction with channel walls. This was accompanied by reduced nuclear solidity and increased structural irregularities, indicative of a more deformable nucleus. These findings were further corroborated in Drosophila models, which showed that ADARB1 depletion induces adjustments in nuclear architecture and an improved migration performance.

Our study positions ADARB1 as a critical regulator of nuclear plasticity and cell motility downstream of E-cadherin loss. These results highlight nuclear mechanotransduction pathways as potential therapeutic targets to limit tumour dissemination.

SYMPOSIUM II
EMERGENT HALLMARKS IN CANCER

Sticky situation: Bacterial-epithelial contact drives colorectal cancer and IgA pathology

Author and Affiliation

Lars Vereecke

CRIG e VIB, Belgium

Abstract

Epithelial barrier stability, maintained by epithelial junctional complexes and the protective mucus layer, is essential for homeostatic host-microbiota interactions. Barrier destabilization promotes pathogenic bacterial-epithelial interactions that drive immune pathology and colorectal cancer (CRC). Using transgenic mouse models of epithelial barrier destabilization, we investigate how impaired barrier integrity shapes pathogenic host-microbe interactions and disease development. Transgenic epithelial expression of Zeb2 induces epithelial-to-mesenchymal transition, enhances bacterial-epithelial interactions and drives spontaneous microbiota-dependent CRC development (Slowicka et al., Nature Cancer 2020). Using this model, we identified the mechanisms by which pks+ E. coli induces epithelial genotoxic stress and CRC progression. We found that pks+ E. coli exacerbates CRC through the type-1 pilus adhesin FimH and the F9-pilus adhesin FmlH. Genetic ablatio of FimH or FmlH, as well as pharmacological inhibition of FimH, reduced bacterial adhesion, attenuated colibactin-mediated genotoxicity and suppresses CRC exacerbation (Jans et al., Nature 2024). In parallel, we developed a transgenic model of mucus barrier disruption by selective depletion of intestinal goblet cells (Muc2DTA mice). Loss of the mucus layer increased bacterial-epithelial interactions and induced epithelial hyperproliferation and colonic polyp formation. Remarkably, Muc2DTA mice also developed hallmarks of IgA nephropathy (IgAN), including elevated serum IgA levels, circulating IgA-IgG immune complexes, and renal IgA deposition. Together, these findings demonstrate that epithelial barrier stability is critical for protection against oncogenic bacterial interactions and systemic immune pathology.

SELECTED ORAL COMMUNICATION

Rab27a Loss Defines an Inflammatory and Therapeutically Vulnerable Subtype of Pancreatic Cancer

Authors and Affiliations

C Dias[1,2,*], N Bastos[1,3], B Adem[4], I Batista[1,5], CF Ruivo[6], S Almeida[1,2], B Morão[7], Guilherme Macedo[2,8], F Carneiro[1,2], M Cravo[9,10], JC Machado[1,2,11], SA Melo[1,2,8]

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10. Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
11. P.CCC – Porto Comprehensive Cancer Center Raquel Seruca

Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, with a 5-year survival rate of 12%. Its aggressive clinical behavior stems from an immunosuppressive tumor microenvironment, and resistance to immunotherapy. Understanding how cancer cells communicate with their microenvironment is essential to identify patient subsets that may benefit from immunomodulatory strategies.

Rab27a is a key regulator of intercellular communication and has been implicated in tumor progression, and therapy resistance. However, its role in shaping the immune-stromal architecture of PDAC remains poorly defined. We generated a GEMM that spontaneously develops PDAC with Rab27a knockout in cancer cells, allowing us to explore how disrupting cancer cell-derived communication alters tumor evolution and microenvironmental composition.

Loss of Rab27a in cancer cells accelerated disease progression and reduced overall survival. Bulk tumor transcriptomic profiling revealed that Rab27a deficiency reprogrammed the tumor microenvironment toward a pro-inflammatory and immune-dependent state, characterized by inflammatory myeloid infiltration, cancer-associated fibroblasts, and production of inflammatory cytokines. Proteomic analysis confirmed enrichment of inflammatory pathways, supporting the emergence of a cytokine-rich

microenvironment which promoted the differentiation of $CD4^+$ T cells toward a Th17-like phenotype. Therapeutic suppression of inflammation, IL-17A neutralization or $CD4^+$ T-cell depletion reduced tumor growth and disrupted this immune program in Rab27a-deficient tumors.

Analysis of human PDAC cohorts identified a subset of patients with absent Rab27a expression and an inflammatory transcriptional profile. These were associated with worse prognosis, defining Rab27a loss as a clinically relevant subgroup for immunomodulatory therapies. This work provides a new framework for patient stratification and precision immunomodulation in pancreatic cancer.

Investigating the role of Dexamethasone in Medulloblastoma progression beyond its anti-inflammatory effects

Author and Affiliation

Adriana Sánchez-Danés

Fundação Champalimaud, Lisbon, Portugal

Abstract

Medulloblastoma is the most common malignant pediatric brain tumor, with the Sonic Hedgehog (SHH) subtype accounting for approximately 30% of cases. SHH medulloblastoma arises from aberrant activation of the SHH signaling pathway in the developing cerebellum and is frequently associated with neurological and motor deficits.

Dexamethasone, a synthetic glucocorticoid, is frequently administered to patients with brain tumors to reduce cerebral edema and intracranial pressure through its potent anti-inflammatory effects. While its clinical use has traditionally been considered purely supportive, our studies using genetically engineered mouse models of SHH medulloblastoma and human organoid cultures reveal that dexamethasone also exerts direct anti-tumor effects, significantly delaying tumor progression.

In this talk, I will discuss the molecular and cellular mechanisms underlying these unexpected tumor-intrinsic effects of dexamethasone and their impact on SHH medulloblastoma growth. Our findings uncover a previously unrecognized interaction between glucocorticoid signaling and tumor progression, challenging the long-held view that dexamethasone functions solely as a supportive therapy in pediatric neuro-oncology.

SELECTED ORAL COMMUNICATION

Macrophage-driven survival pathways enable resistance to radiotherapy in triple-negative breast cancer

Authors and Affiliations

Ana Canha-Borges[1,2,3,4]; Miguel Mendes Ferreira; Bárbara Nunes[1,3,5]; Filipa Leal; Beatriz M Sousa; Flavie Naulin; Winchygn Liu; Joana Lencart[8,9]; Diogo Estêvão[1,2,3,11]; Carmen Jerónimo[12]; Joana Paredes[1,3,5]; Lydia Meziani; Michele Mondini[3*]; Eric Deutsch[3*]; Flávia Castro[1,3*]; Maria J Oliveira[1,2,3*#]

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12. Cancer Biology and Epigenetics Group, Research Center at Portuguese Oncology Institute of Porto (CI-IPOP), CI-IPOP@ RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal;

Abstract

Triple-negative breast cancer (TNBC) is associated with higher risk of relapse following radiotherapy (RT). To overcome this clinical challenge, focus must be paid to immune cells, as macrophages, highly recruited upon RT and associated with tumor progression and recurrence.

Herein, 3D spheroids gathering TNBC cells (tumor spheroids, TS) or TNBC cells with human macrophages (tumor-immune spheroids, TiS) were submitted to five fractions of 5.2Gy, mimicking TNBC patients treatment. Multiomic and in vivo studies were performed to unveil the mechanisms of macrophage-mediated TNBC radioresistance.

Collected data indicates that RT selectively kills cancer cells while does not affect macrophage and cancer cells viability in TiS. Specifically, macrophages protected cancer cells from RT-induced cell death throughout time, promoting their proliferation. Notably, RT seems to promote different macrophage polarization signatures over time, ranging from an anti-tumor pro-inflammatory towards a more pro-tumor

anti-inflammatory phenotype. Concordantly, multiplex ELISA revealed increased secretion of several chemokines and cytokines that potentiate cancer cells survival after RT. Furthermore, in absence of macrophages, irradiated cancer cells displayed increased DNA damage, Ki67 expression and hypoxic cells, as well as ultrastructural cell alterations. Contrarily, irradiated TiS showed low impact of RT and, although not preventing DNA damage after irradiation, macrophages promoted a faster DNA damage repair. In vivo, tumor-associated macrophages (TAMs) blockage significantly repressed orthotopic TNBC mouse tumors growth. Moreover, combining TAMs depletion, but not systemic macrophages depletion, and RT, significantly improved mice overall survival, further suggesting the pro-tumoral role of TAMs in TNBC.
These data confirm that macrophages potentiate TNBC radioresistance through a novel non-autonomous DNA repair mechanism that, when reverted, sensitizes cancer cells to death and improves RT response.

Sensory neurons are needed for both initiation and progression of stomach cancer

Author and Affiliation

Timothy C Wang

Columbia University, USA

Abstract

Cancer cells are now understood to utilize neurons to promote their survival and growth through a variety of mechanisms. Gliomas within the central nervous system (CNS) do this through the establishment of neural circuits and were thought to be distinct from other tumors in this regard. Outside the CNS, peripheral sensory neurons, once considered passive conveyors of nociceptive signals, are now recognized as active and dynamic participants in the progression of solid tumors. We have now shown a comparable pattern of cancer-nerve interactions between the peripheral nervous system (PNS) and gastric cancer (GC). In diverse GC mouse models, nociceptive nerves demonstrated the greatest degree of nerve expansion in an NGF-dependent manner, with CGRP+ peptidergic neurons emerging the primary gastric sensory neurons, as shown through neural tracing. Three-dimensional co-culture models revealed that sensory neurons directly connect through synapse-like structures with gastric cancer spheroids, which upregulate expression of synaptic genes NEUROD1 and ASCL1. Chemogenetic activation of sensory neurons induced the slow release of calcium into the cytoplasm of cancer cells, promoting tumor growth and metastasis. Pharmacological ablation of sensory neurons or treatment with CGRP inhibitors suppressed tumor growth and extended survival. Depolarization of gastric tumor membranes through in vivo optogenetic activation led to sensory nerve depolarization and enhanced calcium flux in jugular nucleus complex, mediated in part through tumor-derived adenosine and glutamine, leading to reflexives CGRP release and defining a cancer cell-peptidergic neuronal circuit. Together, these findings establish the functional connectivity between cancer and sensory neurons in a circuit that may also encompass the tumor microenvironment, identifying this pathway as a potential therapeutic target.

Liquid Biopsies as a Prognostic Factor: Colorectal Cancer as a Clinical Model

Author and Affiliation

Nuno Bonito

Pr. SPO, IPO-Coimbra, Portugal

Abstract

Liquid biopsy has emerged as a transformative approach in oncology, enabling minimally invasive tumour characterisation through circulating biomarkers such as circulating tumour DNA (ctDNA), circulating tumour cells, extracellular vesicles and cell-free RNA. In colorectal cancer (CRC), ctDNA has assumed particular relevance as a dynamic biomarker across the disease continuum, supporting multiple clinical applications from early detection to advanced disease monitoring. This work synthesises evidence from prospective studies and randomised clinical trials, integrating recent data presented at major international congresses, to provide a comprehensive evaluation of ctDNA applications in CRC, including screening, detection of minimal residual disease (MRD), prognostic stratification, treatment adaptation and longitudinal monitoring.

Across the available evidence, ctDNA has emerged as the most clinically validated analyte in CRC, with a robust prognostic impact. Detection of MRD after curative-intent surgery consistently identifies a subgroup of patients at significantly increased risk of recurrence, with markedly inferior disease-free survival outcomes. Serial monitoring of ctDNA further enhances sensitivity and enables real-time assessment of tumour dynamics, offering a window into treatment response and early relapse detection that precedes conventional imaging.

Prospective clinical validation has demonstrated the potential of ctDNA-guided strategies to inform therapeutic decision-making. In stage II disease, biomarker-driven approaches to adjuvant chemotherapy selection have shown the capacity to reduce recurrence risk while minimising overtreatment, establishing proof of concept for personalised treatment strategies. In contrast, in stage III disease, studies exploring escalation and de-escalation approaches have yielded heterogeneous results, reflecting current limitations in assay sensitivity, biological variability and the definition of clinically actionable thresholds.

In metastatic CRC, ctDNA provides a powerful tool for real-time tumour genotyping, enabling detection of emergent resistance mechanisms and dynamic tracking of clonal evolution. These capabilities support more adaptive treatment strategies and are complemented by the development of response assessment frameworks based on liquid biopsy, which may enhance or refine conventional radiological criteria.

Overall, ctDNA is consolidating its role as a key prognostic biomarker in CRC and is progressively transitioning towards integration into routine clinical decision-making. While current evidence strongly supports its use in MRD assessment and risk stratification, widespread implementation of ctDNA-guided treatment strategies requires further prospective validation, improved analytical sensitivity and standardisation across platforms. Future developments are expected to focus on integration with multi-omic approaches, refinement of response criteria and incorporation into biomarker-driven clinical trials aimed at optimising patient outcomes.

Precision medicine in the treatment of lung cancer

Author and Affiliation

João Moreira Pinto

OncMed HLuz, Lisboa, Portugal

Abstract

RLTs in earlier disease setting: current status and future perspectives

Author and Affiliation

James Nagarajah

Radboud University Medical Center, The Netherlands

Abstract

SYMPOSIUM III
CANCER CELL MECHANISMS

Mechano-metabolic adaptations during metastatic dissemination

Author and Affiliation

Vittoria Graziani

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Abstract

SELECTED ORAL COMMUNICATION

Multi-matrix proteomics reveal urine-derived extracellular vesicles as an enriched source of bladder cancer-specific proteins

Authors and Affiliations

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Abstract

Introduction: Current bladder cancer (BlCa) diagnostic and monitoring methods are invasive, highlighting the need for non-invasive approaches. Extracellular vesicles (EVs) are a promising source of novel non-invasive cancer biomarkers. However, isolating BlCa-specific EVs from complex biofluids has been challenging. We address this gap by presenting the first proteomic comparison of EVs across tissue, plasma, and urine from BlCa patients, comparing them to healthy donors (HDs) and multi-cancer controls. Our goal was to pinpoint BlCa-specific biomarkers and better understand EV-mediated communication.

Materials and methods: Using high-throughput mass spectrometry, we analyzed EVs from matched tumor and adjacent normal bladder tissues, as well as plasma and urine samples from the same patients. To ensure robustness and specificity, EVs from plasma and urine of HDs and urine from patients with kidney cancer (ccRCC) and prostate cancer (PCa) were analyzed as comparative controls, enabling identification of BlCa-specific signatures.

Results: The EV proteome was matrix-dependent, unveiling urine as a rich source of potential BlCa-EV biomarkers, presenting 18 upregulated proteins, detected in all tumor samples while absent in HDs' urinary EVs. Matrix comparison provided insights into BlCa communication and enabled tissue-to-urine molecular tracking. Importantly, we identified several candidate biomarkers, exclusively present in BlCa tissue and urine samples. Furthermore, BlCa urinary EV proteins, were absent from all HDs, ccRCC, and PCa samples, demonstrating exceptional BlCa specificity.

Conclusions: This study provides a comprehensive, benchmarked BlCa-EV proteome atlas, identifying novel and highly specific BlCa-EV proteins with strong potential for non-invasive diagnostic and monitoring strategies, contributing to the advancement of precision medicine for BlCa patients.

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Lysosome exocytosis as a therapeutic target in cancer progression

Author and Affiliation

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Abstract

SELECTED ORAL COMMUNICATION

Development of a microRNA-regulated cell death-inducing gene therapy for T-cell Acute Lymphoblastic Leukemia

Authors and Affiliations

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Abstract

Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematological malignancy for which current therapeutic options are limited for relapsed and refractory patients and are associated with severe toxicities. To these unmet needs, we have envisioned a gene therapy (miRGT) that takes advantage of microRNA (miR) post-transcriptional gene regulation and specific expression in cancer to induce the expression of a killing gene selectively in T-ALL cells (miRGToTALL).

Materials and Methods: miRGT consists of a bi-directional lentiviral (LV) vector in which the killing gene is expressed by the EF1α promoter, and the inhibitor of the killing gene promoter is expressed by the PGK promoter. We built miRGT backbone using the Lac operon system as the repressor, by cloning Lac operator sequences (LacO) downstream of EF1α and the Lac repressor (LacI) downstream of PGK. GFP was used as a surrogate marker for the killing gene. Upon generating T-ALL-specific miR profiles by bioinformatics analysis of publicly available miRs datasets, we built several miRGToTALLs with combinations of target sequences (TS) of the miRs whose expression we validated by droplet digital PCR. We cloned in tandem, TS of miRs specifically down-regulated in T-ALL downstream of LacO, and TS of miRs specifically up-regulated in T-ALL downstream of LacI. miRGToTALL specificity to target T-ALL cells only was evaluated by flow cytometry analysis of GFP levels in transduced T-ALL and non-T-ALL cells. We have also validated putative killing genes in vitro.

Results: We have identified two miRGToTALLs with high specificity towards T-ALL cells and a killing gene highly effective in inducing T-ALL cell-death.

Conclusions: Our results demonstrate the feasibility and specificity of miRGT technology. The lead miRGToTALL candidates will now be tested in vitro with the killing gene.

Funding: FCT PhD fellowship (10.54499/2020.08762.BD); ERC-PoC-101069429; CaixaImpulse Health Innovation CI24-20467.

ASPIC – PORTO.CCC RAQUEL SERUCA JOINT SYMPOSIUM – CLINICAL TRIALS DRIVEN BY BIOMARKERS

Genomic guided platform studies Basket of Baskets

Author and Affiliation

Irene Braña

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Abstract

SELECTED ORAL COMMUNICATION

Novel CAR T formulations targeting tumor-associated glycoepitopes: A new strategy for solid tumors

Authors and Affiliations

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Abstract

The accurate targeting of tumor-specific antigens is behind the success of any chimeric antigen receptor (CAR) T cell therapy. While cell surface antigens are rarely exclusively cancer-specific, their post-translational modifications (PTMs) offer a promising alternative. The carbohydrate coat present on every living cell, which is altered in cancer, provides a variety of potential targets for immunotherapeutic approaches. Among these, truncated O-glycans are potential candidates given their prevalence in epithelial tumors and rare detection in healthy tissues.

In this study, we developed a sialyl-Tn (STn)-targeting monoclonal antibody (mAb) with an unprecedented binding profile. We identified the coding sequence of this mAb and engineered its corresponding single-chain variable fragment (scFv) into a second-generation CAR scaffold. The resulting CAR T cells were evaluated in vitro using solid cancer cell models and patient-derived organoids (PDOs), as well as in vivo using human cancer xenograft mouse models.

The mAb specifically binds to epithelial tumors expressing the target antigen without reacting with healthy tissues. The resulting CAR T cells effectively killed solid cancer cell models in vitro. Additionally, these CAR T cells eliminated cancer PDOs while sparing normal organoids. Notably, this CAR T formulation demonstrated robust control over human cancer xenografts in mouse models, underscoring its efficacy in targeting complex solid tumors.

Overall, our study introduces a novel, powerful, and precise glycan-directed CAR T cell therapy designed to target a broad spectrum of carcinomas, combining specificity, efficacy, and a strong safety profile.

Delivering precision immunotherapy with MANIFEST and RISE

Author and Affiliation

Jonathan Lim

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Abstract

SELECTED ORAL COMMUNICATION

Fishing for new immunotherapy compounds to boost innate-tumor rejection

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Abstract

Despite significant advances in cancer immunotherapy, tackling immunosuppression within the tumor microenvironment (TME) remains critical for more effective responses. Using the zebrafish xenograft model, we established that tumor engraftment is governed by innate immunity: some cell lines are recognized and cleared by the host ("regressors"), while others evade it ("progressors"). This platform allowed for an in vivo phenotypic drug screen to identify compounds that boost innate-tumor rejection.

Zebrafish xenografts of human colorectal (CRC) and breast cancer (BC) progressor cell lines were used to screen an FDA-approved small compound library. Tumor cells were injected into 2 days post-fertilization embryos and subjected to compound testing. Tumor clearance was quantified 3–5 days later and hits were defined as compounds that reproducibly increased it.

Screening of >500 drugs identified 23 confirmed hits, several effective in both models. Mechanism of action (MOA) enrichment analysis revealed an overrepresentation of autonomic nervous system-targeting drugs, such as muscarinic receptor antagonists, dopaminergic agonists, and PDE inhibitors. Using transgenic zebrafish lines, we observed the selected hits modulate the innate TME by increasing myeloid cell recruitment and repolarizing macrophages towards a pro-inflammatory phenotype. The top hit, a muscarinic receptor antagonist, was further validated in a CRC mouse model, where it significantly reduced tumor growth as monotherapy and synergized with αPD-1 checkpoint blockade to improve survival. Macrophage depletion in zebrafish and use of NSG mice abolished drug-induced clearance, confirming immune dependence. Ongoing studies include MOA characterization and immune profiling of treated mouse tumors.

We identified a repurposable FDA-approved compound that, when combined with checkpoint blockers to engage both innate and adaptive immunity, may overcome TME-mediated immunosuppression and enhance therapy efficacy.

Towards academic Point-of-Care CAR-T cell therapy in the Netherlands

Author and Affiliation

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Abstract

Recent years have marked a major breakthrough in the treatment of B cell malignancies with chimeric antigen receptor (CAR) T cell therapy. This form of cellular immunotherapy involves the genetic modification of a patient's own T cells, enabling them to specifically recognize and eliminate malignant cells. To achieve this, T cells are collected from the patient, modified and expanded ex vivo, and subsequently reinfused as a therapeutic product. In B cell lymphoma, CAR T therapy has significantly improved survival outcomes and is now reimbursed as standard care.

However, commercial CAR T therapies are associated with several bottlenecks in the Netherlands and across the EU, including long manufacturing times (4–6 weeks) and very high costs, which limit reimbursement and patient access for some products.

In this presentation, I will outline our initiative to address these challenges through academic point of care (PoC) CAR T therapy. Specifically, we have set-up fully hospital based manufacturing, allowing treatment within 7 days at substantially reduced cost. This PoC strategy is currently being evaluated in a phase II non inferiority trial directly comparing it with the commercial CAR T product axicabtagene ciloleucel (Yescarta), with the aim of establishing academic CAR-T therapy as reimbursed care.

Finally, I will discuss emerging CAR T strategies designed for PoC implementation, covering their development from concept and preclinical validation to GMP manufacturing and clinical translation, with examples in T cell leukemia and carcinoma.

POSTERS

01. Functional and molecular characterization of DNMT and HDAC inhibition in NSCLC and RCC cell line models

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Abstract

Introduction: Immune checkpoint inhibitors (ICIs) are standard first-line therapies for advanced cancers such as non-small cell lung cancer (NSCLC) and clear cell renal cell carcinoma (ccRCC). However, only a subset of patients achieves durable responses. Epigenetic therapies have emerged as potential strategies to enhance tumor immunogenicity. DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) can reactivate transposable elements (TEs), releasing immunogenic nucleic acids that trigger antiviral immune pathways through a mechanism known as viral mimicry. In this study, we investigated the cellular and transcriptional effects of DNMTi and HDACi in NSCLC and ccRCC cell models representing different stages of tumor progression.

Materials and Methods: Two NSCLC cell lines (H2087, primary tumor-derived; H2009, metastatic) and two ccRCC cell lines (786-O, primary tumor-derived; Caki-1, metastatic) were treated with the DNMTi and the HDACis. Cell viability assays were used to determine half maximal inhibitory concentration (IC50) values after 48 hours of treatment. Cell cycle distribution and apoptosis were analyzed by flow cytometry, and morphological changes were evaluated by microscopy. Gene expression analyses were conducted to assess epithelial-mesenchymal transition markers and the expression of TEs, including LINE-1 and endogenous retroviruses (ERVs), as well as pathway-level alterations.

Results: All compounds reduced cell viability in a cell line-dependent manner, with IC50 values ranging from 50 nM to 30 μM. Epigenetic treatments induced morphological changes and cell cycle arrest at different phases depending on the drug and cellular context. Increased apoptosis was observed across all models. Gene expression analyses revealed induction of epithelial-mesenchymal transition markers consistent with a hybrid mesenchymal phenotype. In parallel, altered expression of TEs, including LINE-1 and ERVs, was detected. Pathway analyses indicated modulation of proliferative programs together with activation of immune and antiviral signaling pathways.

Conclusions: DNMT and HDAC inhibition induces TEs dysregulation and mesenchymal features in NSCLC and ccRCC cell lines representing different stages of tumor progression. These changes are accompanied by modulation of proliferative and immune-related pathways, supporting the potential of epigenetic therapies to increase tumor immunogenicity.

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02. Defining tumor-associated glycans as oncogenic drivers underpinning therapeutic resistance in ErbB2-positive advanced gastric cancer

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Abstract

Introduction: Advanced gastric cancer (GC) remains a global health concern depicting poor patient prognosis, due to late diagnosis and lack of efficient therapies. Comprehensive molecular profiling of tumors has unraveled possible therapeutic targets, particularly the receptor ErbB2. ErbB2 is overexpressed in 10-20% of advanced GC cases and therapeutically targeted with the trastuzumab (TTZ) monoclonal antibody. However, most TTZ-treated patients develop molecular resistance, leading to disease progression or relapse. ErbB2's extracellular region undergoes extensive glycosylation, which is significantly disrupted in cancer cells. GlycoDisplay aims to understand whether ErbB2 glycosylation actively tunes TTZ binding and its therapeutic efficacy.

Materials and Methods: In vitro characterization of ErbB2 expression levels and activation, and the glycosylation profile of a panel of GC cell lines, by Western blot (WB) and immunofluorescence (IF), prior to cell line selection for CRISPR/Cas9 target glycogene silencing. Establishment of ErbB2-positive glycoengineered GC cell lines depicting pre-determined cell surface glycosylation profiles. Glycophenotyping of glycoengineered cell lines by WB, IF and flow cytometry. Establishment of two ERBB2-amplified TTZ-resistant (TTZ-R) GC cell lines through their continuous exposure to incremental dosages of TTZ, and comprehensive characterization of the resulting glycosylation profile

Results: NCI-N87 and OE-19 cell lines, showing endogenous ErbB2 expression and activation, were selected for CRISPR/Cas9 genetic silencing of the glycosyltransferases ST6Gal1, ST3Gal3, ST3Gal4, and FUT3, which underpin the biosynthesis of several cancer-associated glycans. TTZ-R cell lines depict a reshaping of their glyco-transcriptome and increased ErbB2 activation.

Conclusions: We are setting up a comprehensive cell-based library as an important pre-clinical tool to address glycan-mediated resistance to TTZ in ErbB2-positive GC.

03. Glycosylated Extracellular Vesicles: Key Players in Gastric Cancer Progression and Patient Stratification

Authors and Affiliations

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Abstract

Gastric cancer is a leading cause of cancer-related mortality worldwide, highlighting the need for early diagnostic biomarkers that predict disease aggressiveness and inform treatment decisions. Extracellular vesicles (EVs) are a heterogeneous group of secreted particles involved in intercellular communication, which carry a large repertoire of molecules, including glycoconjugates [1]. The synthesis of short O-glycans is a common feature of tumour cells and EVs and is associated with disease aggressiveness and poor prognosis [2,3,4].

We glycoengineered gastric cancer cells and EVs to investigate the biological impact of short O-glycans on local and distant cell targeting and reprogramming. We have also fully characterized the proteomic and glycoproteomic profiles of these EVs to create a library of putative biomarkers for gastric cancer. We further used a cohort of plasma samples from patients with gastric cancer to validate the identified targets in circulating EVs as sources of molecular biomarkers.

Proteins displaying short O-glycans were identified in EVs isolated from gastric cancer cells and patients' plasma samples. Aberrant glycosylated EVs increased the migration and invasion capacity of recipient cells via activation of NOTCH signalling and drove specific organ tropism toward the liver in mouse models. Short O-glycans were identified in circulating EVs from patients with gastric cancer, whose proteomic content could distinguish non-cancer from cancer individuals, differentiate intestinal from diffuse gastric cancer subtypes, and stratify patients by recurrence risk and prognosis.

Our results clearly demonstrate the significant potential of a multi-omics approach on circulating EVs for cancer biomarker stratification and prognosis. Moreover, our functional in vitro and in vivo investigations strongly indicate that these EVs play a crucial role in the progression of gastric cancer.

References:

[1] Martins ÁM, et al. AdvSci; 2023.

[2] Mereiter S, et al. CancerCell; 2019.

[3] Freitas D, et al. EBioMedicine; 2019.

[4] Freitas D, et al. JEV; 2019.

04. BBIT20-mediated BRCA1–BARD1 disruption overcomes multidrug resistance via mitochondrial collapse in refractory ovarian cancer

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Abstract

Background: Refractory ovarian cancer (OC) is driven by multidrug resistance (MDR) arising from adaptations in DNA repair, transcriptional plasticity, mitochondrial metabolism and drug efflux. As the BRCA1–BARD1 complex is a central regulatory node of these programs, we investigated whether BBIT20, a first-in-class disruptor of this complex, could collapse this resistance network across OC models.

Material and methods: BBIT20 activity was evaluated in OC cell lines, including MDR cells, and patient-derived organoids (PDOs), alone and in combination with paclitaxel (PTX), carboplatin (CB), and PARPi. Mechanistic studies included DNA repair profiling, apoptosis, migration/invasion, P-gp activity, mitochondrial imaging, proteomics and metabolomics. In vivo efficacy and tolerability were assessed in xenograft mice using wtBRCA1 OC cells and mutBRCA1 patient-derived OC cells.

Results: We demonstrate that BBIT20 induces a BRCA status-independent cytotoxicity in OC cells and PDOs (IC50 3–6 μM). In MDR cells, BBIT20 suppressed P-gp expression and reversed EMT. Mechanistically, BBIT20 triggered early BRCA1 depletion followed by RB–E2F and MYC-associated transcriptional remodeling. This culminated in delayed mitochondrial dysfunction, with impaired import, respiratory chain loss and structural disruption. Functionally, BBIT20 restored therapeutic sensitivity to PTX, CB and PARPi in both parental and MDR models (up to 20-fold IC50 reduction) and across clinically resistant PDOs. In vivo, BBIT20 reduced tumor burden and ascitic volume by 75% and, in combination with olaparib, achieved sustained tumor control without systemic toxicity.

Conclusions: BBIT20 dismantles MDR and restores therapeutic vulnerability, representing a promising strategy for refractory OC.

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05. Clarifying the link between deleterious variants in CEP57, ciliogenesis and malignant transformation

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Abstract

Introduction: Prostate cancer (PrCa) is one of the most prevalent malignancies affecting men worldwide. Recently, we reported CEP57, a gene encoding a centrosomal protein, as a potential PrCa predisposing gene. Biallelic pathogenic variants in CEP57 cause mosaic variegated aneuploidy type 2, a rare syndrome characterized by mitotic defects and mosaic aneuploidy, however, a link with cancer development has not yet been established. Considering the role of CEP57 in chromosome segregation and the involvement of other CEP proteins in primary ciliogenesis, we hypothesized that rare CEP57 variants may trigger oncogenic transformation by disrupting primary cilium dynamics.

Materials and Methods: Questioning TCGA PanCancer Atlas Studies (www.cbioportal.org) for tumors carrying CEP57 variants, a cluster of deleterious variants was observed in exon 7, the same affected in the PrCa carrier. To address the oncogenic potential of these variants, we used CRISPR/Cas9 technology to introduce deleterious variants in exon 7 of CEP57 in a non-tumorigenic model of prostate cells. The impact of gene-editing was assessed at transcriptional and proteomic levels, and their biological consequences in cells' proliferation and death ratios was correlated with the impact in primary cilium structure and dynamics.

Results: Deleterious variants in exon 7 led to marked transcriptional CEP57 remodeling, with predominance of alternatively spliced isoforms. These were associated with increased primary cilia frequency and length, and altered basal body organization, which translated into reduced cells' proliferative capacity.

Conclusions: These findings establish a functional link between CEP57 and ciliogenesis, and support a novel mechanism of oncogenic transformation in which truncating variants in CEP57 lead to a trans-differentiation cellular state, characterized by increased primary cilia frequency and stability. Further studies are needed to clarify how this new cellular state may conceal malignant transformation.

06. Multi-omics analysis of TNBC organoids identifies phosphorylation of the membrane trafficking machinery as a key event driving FER-mediated invasion

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Abstract

Triple Negative Breast Cancer (TNBC) is characterised by unfavourable outcome due to its metastatic nature, chemo-resistance, and lack of targeted therapies. Feline sarcoma-related (FER) kinase expression is an independent prognostic factor linked to reduced survival and promotes TNBC invasion by regulating endosomal sorting and recycling (ESR) of adhesion proteins and growth factor receptors. However, ESR mechanisms driving invasion in 3D environments remain unclear.

Using FER-expressing TNBC patient-derived xenograft organoids (PDXOs) and MDA-MB-231 cells, we combined proteomics, phosphoproteomics, and single-cell RNA sequencing to identify key ESR regulators. Invasion in collagen-I correlated with differential phosphorylation of trafficking proteins, such as SEC16A,

and increased Rab4-positive tubules. SEC16A depletion impaired invasion, reducing focal adhesions and Rab4 tubules.

Our results indicate that multi-site phosphorylation of SEC16A is essential for the formation of tubular recycling domains in early endosomes and for subsequent focal adhesion assembly. Importantly, FER controls SEC16A protein levels and localisation specifically in TNBC across multiple models, including 2D cell cultures, in vivo systems, and patient tissues.

Collectively, our findings identify SEC16A as a critical mediator of FER-driven TNBC invasion and highlight membrane trafficking machinery as a promising therapeutic target in TNBC.

07. Mapping Layer-Specific Radiosensitivity in Triple-Negative Breast Cancer Spheroids

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Abstract

Reliable in vitro models to study radiotherapy (RT) response in triple-negative breast cancer (TNBC) remain elusive. While traditional 2D cultures fail to reproduce the complex architecture and heterogeneity of solid tumors, 3D tumor spheroids more accurately recapitulate key features of the tumor microenvironment, as cell-cell interactions and gradients of oxygen and nutrients. These features contribute to the formation of distinct zones within the spheroid closely resembling the in vivo tumor structure and affecting RT efficacy. Herein, we present a 3D TNBC spheroid platform that enables spatial characterization of tumor cell responses to a clinically relevant RT scheme across distinct spheroid regions. MDA-MB-231 spheroids were established and submitted to 5 fractions of 5.2Gy or left without irradiation (CTR). After they were analyzed by proteomics, transmission electron microscopy and immunohistochemistry. Imaging data was after quantified through ImageJ and QuPath and statistical analyzed through GraphPad. RT decreased spheroids viability, while increasing cell size and number of organelles, as mitochondria, lipid droplets, endoplasmic reticulum and autophagic vesicles in surviving cells.

The peripheral layer of the spheroids exhibited the most pronounced organelle alterations and the highest levels of DNA damage, indicated by increased γ-H2AX and CAIX expression, following RT. Proteomic profiling revealed upregulated pathways linked to cell cycle progression and organelle fission, supporting G2/M cell cycle arrest. We hypothesize that, to balance the DNA damage and oxidative stress induced by RT, cancer cells undergo cell cycle arrest, leading to increased cell size and organelle content. Overall, this TNBC 3D model represents a promising platform for radiobiology research and for high-throughput screening of combinatory treatments to overcome radioresistance. As future perspective, we aim to complex these 3D spheroids with stromal populations to mimic closely the tumor microenvironment.

08. Extracellular vesicles enriched in short O-glycans drive tumor–adipocyte crosstalk in gastric cancer

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Abstract

Tumor cells can interact metabolically with adipocytes, which can promote tumor growth and lead to systemic metabolic disorders (1). This bidirectional communication is often mediated by extracellular vesicles (EVs), which act as key carriers of bioactive molecules (2). Glycans are recognized as key regulators of cell–cell interactions within the tumor microenvironment (3,4), yet their role in tumor–adipocyte crosstalk remains largely unexplored. Here, we aimed to clarify the essential role of short O-glycans in facilitating the metabolic interaction between gastric cancer cells and adipocytes, either directly or via EVs.

EVs were isolated from gastric cancer cells with distinct O-glycosylation profiles, and their uptake by adipocytes was assessed by flow cytometry. To measure the capacity of these cells and their derived EVs to induce white adipocyte browning and lipolysis, we performed RT-qPCR, quantified glycerol and fatty acid release, and assessed perilipin immunofluorescence in adipocytes before and after treatment. To assess the feedback impact of beige adipocytes on tumor cells' metabolism and phenotype remodeling, we performed Seahorse metabolic flux assays, fatty acid uptake assays, metabolomic analyses, mitochondrial number quantification, and migration assays.

Our results demonstrated that adipocytes preferentially internalize EVs carrying short O-glycans, which induced adipocyte browning and lipolysis. In turn, as a feedback mechanism, these adipocytes induced a metabolic shift in gastric cancer cells toward fatty acid oxidation, accompanied by increased fatty acid uptake, mitochondrial biogenesis, and enhanced migration capacity.

Together, our findings demonstrate that the presence of short O-glycans in gastric cancer cells and EVs facilitates a bidirectional metabolic crosstalk between tumor cells and adipocytes, highlighting the importance of aberrant glycosylation in cancer-associated metabolic dysfunctions, such as cancer cachexia.

(1) Tisdale MJ. Nat Rev Cancer 2002.
(2) Ramos CC et al. Extracell Vesicles Circ Nucl Acids. 2024.
(3) Mereiter S et al. Cancer Cell. 2019.
(4) Freitas D et al. EBioMedicine. 2019.

09. CDH3/P-cadherin couples hybrid epithelial/mesenchymal plasticity to immune escape in metastatic breast cancer

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Abstract

Epithelial-to-mesenchymal transition (EMT) is a dynamic and reversible program that enables cancer cells to adopt hybrid epithelial/mesenchymal (E/M) states, fostering tumor plasticity and immune evasion, which are two critical features of metastatic progression. However, the molecular mechanisms linking hybrid EMT states to immune escape remain poorly defined. P-cadherin, encoded by CDH3, is a calcium-dependent cell-cell adhesion molecule frequently overexpressed in basal-like and triple-negative breast cancer and associated with poor clinical outcome. Here, we identify P-cadherin as a key regulator connecting EMT plasticity to tumor-immune interactions.

Integrative transcriptomic analyses revealed that CDH3 is enriched in basal-like tumors and Basal A cell lines, partly driven by copy number gains, and correlates with reduced overall survival. CDH3 expression peaks in tumors with intermediate E/M scores and positively associates with both epithelial and mesenchymal gene signatures, implicating it in hybrid EMT states. Furthermore, CDH3-high tumors display enrichment of immune-related pathways, suggesting a functional link between EMT plasticity and immune modulation.

Using an inducible EMT model, we show that EMT progression upregulates P-cadherin and enhances tumorigenic potential, a phenotype recapitulated in stable models with differential P-cadherin levels. Mechanistically, CDH3 upregulation promotes an immune-evasive phenotype with increased PD-L1 expression and reduced MCP-1 secretion, impairing immune cell recruitment and macrophage infiltration in vivo. P-cadherin overexpression attenuates systemic immune activation, as evidenced by reduced splenomegaly and lower neutrophil-to-lymphocyte ratio.

Collectively, our findings establish P-cadherin as a central regulator of hybrid states that links tumor plasticity to immune evasion. These results highlight CDH3 as both a candidate biomarker and a potential therapeutic vulnerability in aggressive metastatic breast cancer.

10. Implications of altered α-Tubulin detyrosination to melanosome biogenesis, melanin synthesis and melanoma

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Abstract

Introduction: Microtubule (MT) post-translational modifications (PTMs) allow for fast and dynamic changes in MT populations, generating a readable code for microtubule-associated proteins (MAPs) and motors that modulate their activity and distribution, and ultimately dictate MT properties and functions. Of all PTMs that can occur in MTs, the detyrosination/re-tyrosination cycle is specific to α-tubulin and has been shown to directly influence dynein- and kinesin-mediated transport, necessary for moving cellular cargoes bidirectionally along MTs.

Methods: Here, we investigated how interfering with MT detyrosination/tyrosination levels, by abrogating tubulin tyrosine ligase (TTL) activity, impacted the distribution of melanosomes, lysosome-related organelles specialized in melanin synthesis present in skin melanocytes. Using a multidisciplinary approach, integrating molecular, cell biology and proteomics analyses in untransformed melanocytes and melanoma cell lines, we unveiled a new and unexpected role of MT detyrosination in melanosome and melanin biogenesis.

Results: Remarkably, loss of TTL in human primary melanocytes leads to a gradual and complete loss of PMEL17, a critical structural protein in melanosomes, and ultimately to the deregulation of the entire melanin biosynthesis pathway, rendering cells amelanotic. This surprising observation could be phenocopied in cells depleted of cytoplasmic dynein, linking the loss of melanosomes and melanin to impaired transport along hyper-detyrosinated MTs. In addition, we found that α-tubulin detyrosination, as well as acetylation, are highly deregulated in melanoma, correlating with aberrant melanin accumulation in patients' primary melanoma samples.

Conclusion: These findings describe a new regulatory pathway for melanosome biogenesis and reinforce the importance of the tubulin detyrosination/tyrosination balance for proper MT-associated cellular functions and its implications in disease processes such as cancer.

11. Unraveling glycosylation dynamics in gastric disease and therapeutic resistance using patient-derived organoids

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Abstract

Altered glycosylation contributes to gastric carcinogenesis and is associated with tumor aggressiveness, poor survival, and therapy resistance. Patient-derived organoids (PDOs) are powerful pre-clinical models that retain key genetic and phenotypic features of their parental tissue. However, their glycosylation profile remains poorly characterized. Here, we validated PDOs as in vivo glycosylation models and identified glycan signatures linked to chemotherapy resistance in gastric cancer (GC).

A gastric PDO biobank (n=56) was established, derived from gastric mucosa of non-tumoral patients (n=11), from adjacent tumor mucosa (n=26), and tumor tissue (n=19) of GC patients1, including sibling PDOs from distinct tumor regions. Omics profiles were analyzed in PDOs and matched tissues, and glycan expression was assessed over time, biobanking, and xenografting. Chemoresistance and CRISPR/Cas9-mediated knockout PDO models were established for functional assays.

PDOs recapitulated the in vivo glycosylation phenotype across the gastric carcinogenic cascade, maintained over time, upon biobanking and xenografting. Lewis type I and II antigens were modulated by PDOs differentiation, aligning with H. pylori's binding patterns and mirroring tissue interactions. Glycosylation was remodeled during the acquisition of chemoresistance, specifically the loss of FUT8-mediated core fucosylation. FUT8 knockout promoted therapy resistance compared with FUT8-positive PDOs. Loss of core fucosylation was associated with poor clinical outcomes and disease recurrence in GC patients.

These findings establish PDOs as a valuable platform to investigate glycosylation-driven chemoresistance and identify core fucosylation as a clinically relevant biomarker and potential therapeutic target in chemotherapy-resistant GC.

1Santos-Ferreira, Liliana et al. “Patient-derived organoids to study glycosylation dynamics during gastric disease.” Cell reports vol. 44,11 (2025): 116550. doi:10.1016/j.celrep.2025.116550

12. Decoding the molecular dynamics of Heparan Sulfate and Chondroitin Sulfate expression along the gastric carcinogenesis

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Abstract

During gastric cancer development, several molecular changes drive cellular malignancy and disease progression, with aberrant glycosylation emerging as a key feature impacting cellular functions. While many studies have described changes in N- and O-linked glycans in the gastric mucosa during carcinogenesis, changes in glycosaminoglycans remain comparatively less studied.

Altered expression of Heparan Sulfate (HS) and Chondroitin Sulfate (CS) glycosaminoglycan chains has been associated with biosynthetic dysregulation, leading to structural and functional changes on HS/CS proteoglycans. Recently, our group has shown that Syndecan-4 proteoglycan expression is linked to gastric cancer cells malignant features, and associates with poor survival of gastric cancer patients. However, HS/CS molecular changes within the gastric cancer cascade are underexplored. The understanding of the HS/CS molecular dynamics during gastric cancer progression sets the ground for the identification of specific glyco-signatures with diagnostic and prognostic potential. In this work, we aim to disclose glycosylation changes in HS/CS carriers during gastric carcinogenesis and reveal their impact on cell behavior and cancer progression.

HS, CS and proteoglycans (PGs) expression profiles were analyzed in clinical samples by in situ immunolabelling and structural disaccharide profiling. Gastric cancer cell lines were genetically glycoengineered to HS/CS profiles found in patients and characterized regarding glycosaminoglycan and PG profiles. HS/CS signatures' impact on tumorigenesis was explored by cellular and in vivo functional assays. Different stages of the gastric cascade showed distinct HS/CS/PGs signatures, with specific HS/CS-sulfation trends in tumors compared to healthy mucosa. Functionally, the motility of cancer cells was impacted by HS/CS loss. Overall, our results showed unique HS/CS expression and sulfation trends across the gastric cascade, with impact in cell aggressive features.

13. Impact of sialylation inhibition on Cetuximab therapy in colorectal cancer

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Abstract

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related death worldwide. Aberrant sialylation is a hallmark of cancer, contributing to tumour progression, immune evasion, and resistance to therapy. However, its impact on the efficacy of EGFR-targeted treatments remains unclear, particularly in the context of resistance to Cetuximab. This study aims to evaluate how sialylation inhibition affects malignant features of CRC cells and the response to Cetuximab therapy.

Methods: Three RAS wild-type CRC cell lines with distinct sialylation patterns- SLeX, terminal α2,6 sialylation, and ST6GalI overexpressing model- were treated with the pan-sialyltransferase inhibitor 3Fax-Neu5Ac, to assess changes in sialylation, associated biological effects, and therapeutic response.

Results: Treatment with 3Fax-Neu5Ac led to a marked reduction in both α2,3- and α2,6-terminal sialylation at the cell surface. N-glycomic profiling revealed a remodeling of the glycome, with a decrease in sialylated glycans and an increase in neutral structures. This sialylation inhibition didn't affect viability but significantly impaired cell motility. While total receptor and basal activation remain unchanged, inhibition of sialylation altered the glycosylation profile of EGFR, particularly affecting terminal α2,6-sialylation. Notably, cells enriched in α2,6-sialylation displayed altered Cetuximab binding.

Conclusion: Overall, these findings demonstrate that EGFR sialylation plays a critical role in modulating receptor response to Cetuximab in CRC cells. Targeting sialylation emerges as a promising strategy to overcome glycosylation-driven therapeutic resistance.

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14. BBIT20 enhances immunotherapy response in a preclinical model of pancreatic cancer

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Abstract

Introduction: Pancreatic cancer (PC) is among the deadliest malignancies. Its desmoplastic and 'immune-cold' microenvironment drives marked therapy resistance, particularly to immunotherapies. Targeting the homologous recombination (HR) DNA damage repair pathway has been shown to improve immunotherapy responses in PC by enhancing tumour immunogenicity. Here, we evaluated the antitumoral activity of BBIT20 – a first-in-class HR inhibitor – in a syngeneic PC model, as a monotherapy and combined with anti-PD-1 immunotherapy, and explored its impact on the tumour immune landscape.

Materials and Methods: C57BL/6J mice were subcutaneously injected with KPC pancreatic cancer cells and treated with vehicle, 2 mg/kg of BBIT20, 100 µg/animal anti-PD-1, or the corresponding combination therapy. Tumour growth was monitored weekly, and tumours were collected at endpoint. Immunohistochemistry was conducted to assess the levels of PD-L1, BAX, VEGF, Ki-67, and other immune biomarkers.

Results: BBIT20 elicited a stronger antitumor effect than anti-PD-1. Moreover, it significantly enhanced the immunotherapy response, suggesting a synergistic interaction. Tumours treated with BBIT20 or the combination displayed significantly reduced expression of PD-L-1, VEGF, and Ki-67, and increased BAX levels, when compared with the other groups. Additional analyses are ongoing to characterize tumour immune cell infiltration and activation.

Conclusions: BBIT20 enhanced the antitumor efficacy of anti–PD-1 in pancreatic cancer, highlighting its potential to increase tumour immunogenicity and thereby improve responses to immunotherapy.

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15. Mechanistic insights into BBIT20 interaction with BRCA1: a first-in-class approach to overcome cancer resistance

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Abstract

Introduction: Despite significant advances in oncology, aggressive malignancies such as pancreatic cancer (PC) remain highly resistant to current therapies. Tumors harbouring deficiencies in the homologous recombination (HR) DNA damage repair pathway display increased vulnerability to therapeutic interventions. However, such defects occur in only a small subset of PC patients, underscoring the need for alternative strategies. BBIT20, a first-in-class inhibitor of the BRCA1/BARD1 heterodimer, has shown potent antitumor activity in PC models, both alone and combined with chemotherapy. Computational analyses suggest preferential engagement of the BRCA1 RING domain, yet the mechanistic details of the BBIT20-BRCA1 interaction remain to be experimentally defined.

Methods: Escherichia coli BL21 (DE3) was used for recombinant BRCA1 RING domain production and subsequent purification. Fluorescence quenching and far-UV circular dichroism were performed to analyse the interaction between BBIT20 and BRCA1.

Results: Increasing concentrations of BBIT20 induced progressive quenching of BRCA1 RING domain fluorescence. Complementary circular dichroism results showed concentration-dependent changes in secondary structure, indicating the binding of BBIT20.

Conclusions: This work demonstrates that BBIT20 directly targets the BRCA1 RING domain, inducing conformational changes that disrupt the BRCA1/BARD1 interaction and thus compromise HR pathway. Ongoing isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) studies will further refine our understanding of the molecular binding of BBIT20 to BRCA1, guiding the development of optimized BBIT20 derivatives.

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16. Gastric carcinogenesis modelling using glyco-engineered patient-derived organoids

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Abstract

Introduction: Gastric cancer (GC) remains the 4th leading cause of cancer-related deaths worldwide. It develops through a cascade of progression steps, which are marked by key alteration events. Among these, the early p53 dysfunction and aberrant expression of sialylated structures and truncated O-glycans stand out. Despite being well described, its precise triggers and mechanisms remain unclear, partly due to the lack of accurate in vitro models that recapitulate carcinogenesis overtime. Recently, we validated patient-derived organoids (PDOs) as reliable ex vivo models to study gastric disease, including the study of glycan profile over the GC cascade (Santos-Ferreira et al., Cell Reports, 2025).

Materials and Methods: To address the role that glycans play in carcinogenesis, as well as their potential interplay with oncogenes, we generated engineered PDOs mimicking early GC events, such as TP53 loss, using a lentiviral system for CRISPR-Cas9 gene editing. Its impact will be evaluated histologically and molecularly, namely through the expression of cancer-associated markers and changes in the glyco-profile.

Results: A shift in dysplastic-like PDO's phenotype was observed alongside with the upregulation and de novo expression of cancer-associated glycans, suggesting a close link between glycosylation and oncogene regulation.

Conclusion: The establishment of such engineered advanced ex vivo models provide a powerful platform to dissect the molecular mechanisms underlying GC initiation and support the development of improved biomarkers for early detection and preventive strategies for patients.

17. Disruption of Cell-Cell Adhesion Activates the Integrated Stress Response and Stress Granules Formation in Inflammatory Breast Cancer

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Abstract

Introduction: Inflammatory breast cancer (IBC) is an aggressive malignancy characterized by rapid progression, poor prognosis, and limited therapeutic options. An IBC hallmark is the formation of tumor emboli, which are clusters of circulating tumor cells with enhanced metastatic capacity and stem-like properties. Cell-cell adhesion, primarily mediated by cadherins, plays a critical role in maintaining these structures. However, the consequences of adhesion loss on tumor cell survival and stress adaptation remain unclear.

Methods: E- and P-cadherin were silenced using RNAi and CRISPR/Cas9 in IBC cell lines. The effects were assessed in 3D spheroids, focusing on cell viability, ROS levels, and signaling pathways. Proteomic profiling, expression analyses and electron microscopy were used to characterize stress responses and cellular adaptations.

Results: E- and P-cadherin were confirmed as mediators of cell-cell adhesion in IBC. However, their loss did not significantly impair spheroid survival or alter ROS levels. Instead, proteomic analysis revealed activation of the integrated stress response (ISR), marked by robust stress granules (SG) formation and upregulation of SG-associated proteins, like G3BP2. These findings were validated at both transcript and protein levels and supported by ultrastructural analysis. Loss of adhesion was also associated with increased expression of EMT and stemness-related genes, as well as enhanced metabolic activity, as indicated by elevated lipid droplet accumulation and mitochondrial content. Importantly, pharmacological inhibition of ISR signaling or G3BP function significantly reduced metabolic activity in adhesion-deficient IBC spheroids.

Conclusion: Loss of cell-cell adhesion in IBC does not compromise survival, but promotes a stress-adaptive state driven by ISR activation and SG formation. Targeting this pathway, particularly in combination with strategies that disrupt cell–cell adhesion, represents a promising therapeutic approach for IBC.

18. Tumor organoid-based 3D models to uncover predictive biomarkers for breast cancer progression

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Abstract

Introduction: Breast cancer (BC) is the most common cancer in women and, despite continuous research, its relatively high mortality due to metastasis remains a major concern. Prognosis varies by subtype: Luminal A and B, HER2-overexpression and Triple-Negative (including Basal-like and Claudin-low), the latter two having the worst outcomes. Identifying predictive biomarkers of metastasis is crucial to improve prognosis. While traditional 2D models fail to replicate tumor microenvironment complexity, 3D tumor-derived organoids mimic better the in vivo conditions. Thus, we aimed to develop and optimize a protocol to generate BC organoids from xenografted tumors representing different subtypes and then to adapt it for patient samples.

Material & Methods: We conducted a pre-clinical study by injecting BC cell lines representing each subtype (MCF-7/Az and T47D for Luminal A; BT474 for Luminal B; SKBR3 and MDA-IBC-3 for HER2+; BT20 and MDA-MB-468 for Basal-like; MDA-MB-231 and SUM149PT for Claudin-low) into immunosuppressed female N: NIH(S)II-nu/nu mice. After tumor growth, tumors were excised and processed to establish tumor-derived organoids. A non-tumorigenic human cell line (MCF10A) and mouse breast samples were used as healthy controls. After optimization, the protocol was applied to primary breast tumor samples from IPO-Porto to generate patient-derived organoids (PDOs).

Results: Both cell line-derived and patient-derived organoids showed success rates consistent with the literature (~70% and ~55%, respectively). Furthermore, organoids retained the histological profile of their original tumors, supporting their value for studying metastatic predictive biomarkers. While further analyses are ongoing, this study lays groundwork for future research.

Conclusions: Organoid cultures provide a more biologically relevant model for tumor biology. Their successful establishment opens opportunities to study BC progression, potentially improving early detection, treatment and patient prognosis.

19. Assessing the potential of specific tubulin PTMs as predictive biomarkers for taxane response in breast cancer

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Abstract

Introduction: Taxanes are widely used in neoadjuvant chemotherapy (NAT) for breast cancer (BC) acting by stabilizing microtubules. Despite their clinical success, response rates vary considerably, and side effects impose a significant burden, highlighting the need for reliable predictive biomarkers. We previously showed that 2 tubulin post-translational modifications (PTMs) — α-tubulin acetylation and detyrosination — correlate with or influence taxane response in vitro. Here, we conducted a retrospective study evaluating these PTMs in BC patients treated with taxanes.

Materials and Methods: We established an immunohistochemistry (IHC) protocol and scoring system to quantify α-tubulin acetylation and detyrosination in formalin-fixed paraffin-embedded (FFPE) tissue. Tumour biopsies from 60 BC patients (all subtypes) undergoing taxane-based NAT were collected alongside clinical and histopathological data. Post-NAT surgical specimens were also analyzed when available. PTM levels were evaluated by three independent pathologists and digital image analysis, generating a final AcScore reflecting intensity, extension and homogeneity, used for assessing the correlation with therapeutic response.

Results: Clinical nodal stage emerged as the strongest predictor of NAT response in this cohort. α-tubulin acetylation levels (AcScore) were not significantly associated with therapeutic response in univariable or multivariable analyses. Notably, pathologist assessment and digital quantification of acetylated α-tubulin showed strong concordance, demonstrating the robustness of this approach for clinical evaluation.

Conclusions: We established a robust tool to assess tubulin PTMs in FFPE material. This study highlights the need for refined in vitro models, such as patient-derived organoids, to evaluate the predictive value of tubulin PTMs in taxane response — laying the groundwork for personalized dosage optimization and opening new avenues for targeting tubulin PTMs as therapeutic strategies in BC.

20. BCL6 expression may predict outcomes in Luminal A breast cancer: evidence from public genomic cohorts

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Abstract

Introduction: Breast cancer (BC) is the most common malignant neoplasm among women worldwide and remains a leading cause of cancer-related mortality. It is a heterogeneous disease classified into molecular subtypes with distinct prognostic and therapeutic implications. Luminal A is the most prevalent subtype, characterized by high expression of hormone receptors (estrogen and progesterone), low proliferation rate, and generally favorable prognosis. However, consistent evidence indicates a significant risk of late recurrence and new neoplastic events, posing challenges for clinical follow-up strategies.

Material and Methods: We analyzed genomic data from the TCGA breast cancer cohort (n = 1247) to assess the prognostic value of the BCL6 gene, a transcriptional regulator previously implicated in tumor progression. Data on BCL6 expression, molecular subtyping (PAM50), and overall survival (OS) were retrieved.

Results: Although BCL6 expression was globally reduced in tumors compared to normal tissue, it was significantly higher in Luminal A tumors than in other subtypes, with a subgroup (44%) maintaining expression levels similar to normal tissue. Importantly, within the Luminal A subtype, higher BCL6 expression was associated with poorer long-term survival ($p = 0.041$).

Discussion: These findings support the potential of BCL6 as a stratification biomarker for the risk of long-term neoplasm recurrence within Luminal A breast cancer, with possible implications for tailoring the intensity and duration of clinical follow-up.

21. What is the role of Pathology staging in improving Pre-Treatment Risk Stratification in Prostate Cancer for biomarker selection?

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Abstract

Prostate cancer (PCa) remains the 2nd most common cancer in men with ~35k deaths (projected USA/2025) due to advanced and metastatic disease. The most used tools for risk stratification rely on pre-treatment variables such as PSA, clinical T stage (cT), and biopsy's Gleason Score (GS), which are often inaccurate. Efforts have been made to improve risk stratification, such as Cambridge Prognostic Group (CPG) model, which outperforms D'Amico-based systems, although it still relies on these imperfect surrogates. In this study, we aimed to validate a new tool, replacing cT stage by pathological T stage (pT) and using GS from surgery as a reference for biomarker assessment, as a better tool to the development of more clinically meaningful liquid biopsy-based biomarkers.

A cohort of 157 patients treated for PCa at IPO Porto between 1999-2010 was randomly selected (CES 175/022) and data, such as PSA at diagnosis, cT, and pT, as well as the GS of the biopsy and specimen, were retrieved from the clinical charts. Descriptive data, comparisons, and survival analysis were assessed. Most of the patients were categorized as a non-clinically significant PCa (low risk and favorable intermediate risk) – 74.5% – based on CPG tool, while the same categories only represent 47.8% based on IPO Porto tool. Additionally, based on biopsy, 102 patients were classified with a ISUP 1, while only 66 were classified as the same group based on the specimen. Regarding recurrence prediction, the IPO Porto tool showed high accuracy (80.25%) while the CPG tool only showed 66.24%.

Moreover, the IPO Porto tool achieved the highest and most consistent concordance index across all time points, outperforming both clinical and pathological classifiers.

In conclusion, our results showed a reinforced potential of IPO Porto tool as a robust reference standard for developing liquid biopsy-based biomarkers for pre-treatment PCa risk stratification.

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22. Genomic Landscape and Gene Silencing to Overcome Therapeutic Resistance in Pancreatic 3D Models

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Abstract

Pancreatic Ductal Adenocarcinoma (PDAC) is characterized by a dismal prognosis, largely driven by profound intrinsic chemoresistance and a dense desmoplastic stroma that acts as a major physical barrier to therapy. To faithfully recapitulate this complex tumor architecture and clinically relevant drug responses, we utilized advanced 3D in vitro models, specifically PANC-1 spheroids and patient-derived organoids (PDOs), which were initially established and comprehensively validated through phenotypic monitoring and mutational profiling.

To further elucidate the mechanisms driving therapeutic refractoriness, a delivery system based on functionalized gold nanoparticles, designated as gold nanobeacons (AuNBs) was used for the targeted silencing of a resistance-associated transcript. This AuNB-mediated suppression was successfully implemented in both the PANC-1 spheroids and the PDOs. Following efficient silencing of the molecular target, the 3D models were subjected to standard-of-care chemotherapeutic regimens currently employed in the clinical management of PDAC.

The efficacy of this combinatorial approach was quantitatively evaluated through cell viability assays. Our results demonstrate that the nanobeacon-induced suppression of this factor significantly enhances the pharmacological response in both model systems. Notably, the intervention effectively diminishes intrinsic chemoresistance, resensitizing both the cell line-derived spheroids and the PDOs to the cytotoxic effects of the clinical regimens.

These findings suggest that the disruption of this specific molecular vulnerability via functionalized AuNBs serves as a powerful synergistic strategy to overcome therapeutic resistance and improve clinical outcomes in PDAC.

23. Predicting response to neoadjuvant therapy in locally advanced rectal cancer

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Abstract

Rectal cancer is the 8th most common neoplasia worldwide and accounts for 3.5% of cancer-related fatalities. The standard treatment for locally advanced rectal cancer (LARC) includes neoadjuvant therapy (radiotherapy with or without chemotherapy) followed by resection surgery. However, patients exhibit highly variable responses to neoadjuvant treatment, ranging from complete response to no tumour regression.

Currently, there are no clinically validated approaches to predict response, and neoadjuvant treatment is administered to most patients without knowing whether it will provide meaningful benefit. Accurate predictive biomarkers would thus be very useful to stratify LARC patients, identifying those likely to achieve a complete response and those unlikely to respond, who may be better suited for immediate surgery, being spared from unnecessary toxicity and the risk of disease progression during ineffective treatment.

Given this unmet need, we have analysed publicly available Affymetrix microarray data comprising pre-treatment biopsy gene expression profiles of 13 LARC patients, as well as matched clinical data, including response to neoadjuvant therapy. These biopsies were previously processed by laser capture microdissection to separate stroma and epithelium for tissue-specific analyses. Using Lasso regression, we were able to derive a response score based on the weighted expression of candidate genes in the tumour stroma. This score successfully separated LARC patients according to their response to neoadjuvant therapy, and a permutation analysis supported its robustness.

These findings, however, are based solely on computational analyses of a third-party dataset. Experimental validation in an independent patient cohort is being planned to confirm their predictive value.

24. Gastric cancer organoid as model for therapy resistance

Authors and Affiliations

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Abstract

Introduction: Gastric cancer (GC) remains a major global health burden and a leading cause of cancer-related mortality. Despite advances in treatment, the prognosis for advanced disease is poor, with a 5-year survival rate of 20–40%. Combination chemotherapy regimens such as FLOT are the current standard of care; however, responses vary, and resistance often develops. Increasing evidence suggests that aberrant glycosylation plays a key role in GC progression, immune evasion, and therapy response. Patient-derived organoids (PDOs) provide a physiologically relevant ex vivo model to study tumor biology and treatment responses. This study aimed to establish GC PDO models and investigate chemotherapy-induced changes in glycosylation profiles.

Methods and Results: Five GC PDO models were established from primary tumors (n=3) and matched adjacent mucosa (n=2). Normal PDOs displayed spherical and cystic morphologies, while tumor PDOs showed more complex structures resembling the original tumor histophenotype. All organoids demonstrated sustained proliferative capacity, enabling comparative studies within the same patient context. Two intestinal-type tumor PDOs were treated with varying concentrations of FLOT (5-FU, leucovorin, oxaliplatin, docetaxel). Dose–response analysis revealed a dose-dependent reduction in cell viability. IC_{50} values were derived from FLOT response curves and used to define optimal treatment conditions. Glycomic profiling showed chemotherapy-induced remodeling, with increased truncated O-glycans and sLe^a and decreased sLe^x. Receptor tyrosine kinase analysis indicated altered MET and HER2 expression following treatment.

Conclusion: Chemotherapy induces glycoprofile remodeling and modulates signaling pathways in GC PDOs. These findings support the use of PDOs to study glycosylation-mediated chemotherapy responses and may aid in identifying glycan-based biomarkers and therapeutic targets for precision oncology in GC

25. Triple-negative breast cancer shapes bone marrow differentiation by favoring plasmacytoid dendritic cells in Flt3L-responsive precursors

Authors and Affiliations

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Abstract

Introduction: Cancer is a systemic disease affecting distant organs beyond its primary site, such as the bone marrow (BM), via soluble factors and extracellular vesicles, including exosomes. These factors reach the BM, which contain the dendritic cells (DCs) immune precursors and impair differentiation and function of BM-DCs ex vivo in healthy mice. Here, we characterised BM-DCs generated from mice with orthotopic or metastatic breast cancer.

Materials and Methods: 20-24-week-old female BALB/c mice were injected orthotopically or intravenously with 4T1-Luc2+ cells. BM cells were isolated, differentiated and matured into DCs, and analysed by flow cytometry. Cytokines were quantified in supernatants.

Results: Tumour-bearing mice exhibited a shift in BM-DC subset composition, with an increased proportion of plasmacytoid dendritic cells (pDCs) relative to conventional DCs. Mature BM-DCs from tumour-bearing mice showed reduced expression of CD40 and CD86, while H-2 expression showed a decreasing trend compared to controls. Cytokine profiling revealed an increased IL-12(p70)/IL-10 ratio and elevated IL-6 levels.

Conclusions: Overall, these findings indicate expanded pDC development in tumour-bearing mice and suggest that, despite increased maturation-associated activation, BM-DCs remain functionally impaired in priming anti-tumour T cell responses, facilitating immune evasion and tumour progression.

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26. Liquid Biopsy Insights: Urinary EV microRNAs for Bladder Cancer Detection

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Abstract

Introduction: Bladder cancer (BlCa) : cystoscopy and urine cytology have limited sensitivity for BlCa detection reinforcing the investigation on new biomarkers. No FDA/CE-approved biomarkers currently allow for aggressive disease (muscle invasive (MIBC)) triage. Extracellular vesicle (EV)-derived miRNAs have been proved as potential biomarkers in cancer. Herein, we profiled and tested urinary EV-miRNAs value for BlCa detection and BlCa aggressiveness triage biomarkers.

Materials and Methods: Urine EVs were separated and EV-RNA was isolated. EV-derived miRNAs from BlCa patients and healthy donors (HDs) were profiled using the TaqMan Low-density Arrays (TLDAs) to find biomarkers for BlCa detection. The most promising candidates were assessed in 20 HDs and 102 BlCa patients (IPOPortoFG-CES_221/020) by droplet digital PCR. A logistic regression nomogram was done including age, gender and miRNAs levels with evaluation of performance by Youden index.

Results: Twenty nine miRNAs showed differentially expressed levels between HDs and BlCa. The top 3 overexpressed miRNAs in BlCa were tested in an independent series and their levels correlated with gender and age. Since the tested miRNA levels were gender and age dependent, a nomogram was created combining miRNAs levels, gender and age showing 76% sensitivity and 95% specificity (AUC=0.87) detecting BlCa. A nomogram panel containing miRNAs levels and gender detected MIBC with 86% sensitivity and 61% specificity (AUC=0.73).

Conclusions: The BICa detection nomogram might be a valuable biomarker, outperforming urine cytology (55% sensitivity and 92% specificity), the current gold standard alongside cystoscopy. A nomogram for MIBC detection also showed promising performance and may help triage patients with aggressive disease for earlier TURBT, enabling faster histological confirmation and treatment.

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27. Enhancing standard therapy efficacy in triple-negative breast cancer by targeting BRCA1–BARD1 interaction

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Abstract

Triple-negative breast cancer (TNBC) is an aggressive, early onset cancer marked by metastasis, high recurrence, and limited treatment options. Current management relies on chemotherapy-based multimodal approaches with limited benefit and significant toxicity. Targeting homologous recombination (HR) DNA repair is a promising strategy, with PARP inhibitors (PARPi) approved for mutant (mut) BRCA TNBC. Still, resistance and low prevalence of mut BRCA limit their clinical benefit, underscoring the need for strategies that improve response rates in TNBC. Our group identified BBIT20, the first inhibitor of the BRCA1-BARD1 interaction that disrupts HR, inducing cancer cell death independently of BRCA status, in pancreatic and ovarian cancers. This work evaluated the potential of BBIT20 to enhance standard therapy efficacy in TNBC. Antiproliferative activity of BBIT20 was assessed in wild-type and mut BRCA TNBC cells by the sulforhodamine B (SRB) assay, upon 48 h of treatment. Its effect was compared with conventional (paclitaxel and carboplatin) and targeted (olaparib and talazoparib) therapies. Clonogenic assays evaluated long-term effects on colony formation. Combination treatment with standard therapies was analyzed by SRB assay, and apoptosis induction by flow cytometry.

BBIT20 showed promising antitumor activity in TNBC cells, compared to standard therapies, and maintained activity independently of BRCA mutational status, unlike PARPi. Preliminary data suggest that BBIT20 sensitize TNBC cells to paclitaxel, carboplatin, and PARPi, synergistically enhancing apoptosis and supporting its application in combination therapy.

Further work is underway to demonstrate its potential as an anticancer drug candidate in TNBC treatment.

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28. RKIP regulates metabolic reprogramming and sensitizes triple-negative breast cancer to OXPHOS inhibition

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Abstract

Introduction: Metabolic reprogramming is a well-established hallmark of cancer and plays a crucial role in metastatic progression. Triple-negative breast cancer (TNBC) is an aggressive subtype with high metastatic potential. However, the role of metastasis suppressors such as Raf Kinase Inhibitory Protein (RKIP) in regulating tumor metabolism remains unclear.

Methods: Metabolic and functional analyses were performed in BM1 TNBC cells overexpressing RKIP and in orthotopic xenografts. Mitochondrial activity, metabolic contributions, and lipid abundance were assessed using Seahorse analysis, mass spectrometry, and [U-^{13}C]-glucose stable isotope tracing. Transcriptomic changes were analyzed by bulk and single-cell RNA sequencing. Sensitivity to the OXPHOS inhibitor metformin in vivo, and associations between RKIP expression and metabolic signatures in patient datasets, were evaluated.

Results: RKIP overexpression induced a shift toward oxidative metabolism, with increased mitochondrial metabolites, respiration, and fatty acid oxidation dependency. In vivo, RKIP-expressing tumors showed increased glucose contribution to the TCA cycle metabolite citrate and reduced free fatty acid levels. Transcriptomic analyses revealed enrichment of oxidative phosphorylation (OXPHOS) pathways and reduced variability in OXPHOS gene expression upon RKIP overexpression in tumors. RKIP-overexpressing tumors were more sensitive to OXPHOS inhibition by metformin. In patient datasets, RKIP expression correlated with OXPHOS signatures, and RKIP-promoted gene signatures were associated with improved distant metastasis-free survival.

Conclusions: RKIP promotes mitochondrial oxidative metabolism in TNBC and is associated with distinct metabolic dependencies. These findings link metastasis suppression with metabolic regulation and support further exploration of OXPHOS-targeted therapeutic strategies.

29. Uncovering drug resistance in colorectal Cancer through EV profiling in 2D and 3D systems

Authors and Affiliations

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Abstract

Colorectal cancer (CRC) remains a major cause of cancer-related mortality, with tumor heterogeneity and multidrug resistance (MDR) limiting therapeutic efficacy. Extracellular vesicles (EVs) mediate intercellular communication within the tumor microenvironment, contributing to progression and resistance. Three-dimensional (3D) models, such as spheroids and organoids, better recapitulate tumor complexity than conventional two-dimensional (2D) cultures. This study investigated EV-associated features in CRC resistance and the impact of 2D and 3D models.

CRC cell lines, including doxorubicin- and oxaliplatin-sensitive and -resistant models, were cultured in 2D and as 3D spheroids. EVs were isolated and characterized by nanoparticle tracking analysis and protein quantification. Supernatants were analyzed by Fourier-transform infrared spectroscopy (FTIR), and RNA sequencing (RNA-seq) assessed molecular differences associated with resistance and culture dimensionality.

Resistant models showed distinct EV-associated profiles compared to sensitive cells, with differences between 2D and 3D conditions. EV characterization revealed variations in particle distribution and protein content, while FTIR indicated compositional differences in supernatants. RNA-seq identified differential molecular signatures associated with resistance.

These findings support the relevance of 3D models in recapitulating tumour behaviour and suggest that EV-associated features reflect resistance mechanisms, with ongoing application to patient-derived organoids reinforcing their potential as CRC biomarkers.

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30. Sequential siAKT2 nanoparticle dosing induces transient transcriptional disruption followed by adaptive recovery

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Abstract

Lung cancer remains the leading cause of cancer-related mortality worldwide, highlighting the need for more effective and targeted therapeutic strategies. The efficacy of RNA interference (RNAi)-based nanotherapies is critically influenced by dosing strategies and cellular adaptation. Here, we investigated the transcriptomic response of A549 non-small cell lung cancer cells following sequential administration of polymeric nanoparticles delivering siRNA targeting AKT2.

Transcriptomic profiling revealed a dose-dependent but non-linear response, with modulation of pathways associated with stress response and survival. Following the first dose, moderate alterations in signaling pathways were observed. In contrast, the second dose induced a marked and widespread disruption of gene expression, characterized by global attenuation of multiple cellular pathways, indicating a strong perturbation of cellular homeostasis. This state could not be clearly attributed to canonical processes such as proliferation arrest or apoptosis based on transcriptomic signatures alone. Following the third dose, partial restoration of transcriptional activity was observed, accompanied by reactivation of pathways associated with survival and adaptive responses, suggesting the emergence of a resistant phenotype.

Overall, these findings demonstrate that repeated siRNA nanoparticle delivery does not produce cumulative effects but instead drives dynamic and stage-specific cellular responses. The identification of a transient disruption state highlights a potential therapeutic window for optimizing RNAi-based nanomedicines in lung cancer.

Keywords: RNA interference; Nanoparticles; Lung cancer; Transcriptomics; Drug resistance; Dosing strategy

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31. Epigenetic drugs differentially modulate transposable element expression in NSCLC models: comparative analysis across three TE quantification pipelines

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Abstract

Introduction: Lung cancer is the leading cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) accounts for 85% of cases. Epigenetic drugs, such as DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), can remodel the tumor epigenome and modulate transposable element (TE) activity, potentially contributing to tumor immunogenicity. This study aimed to characterize the effects of DNMTi and HDACi on TE regulation in two NSCLC in vitro models using three bioinformatic pipelines.

Methods: NCI-H2087 (primary) and NCI-H2009 (metastatic) NSCLC cell lines were treated with Azacitidine (DNMTi) and Panobinostat (HDACi) at their IC50 for 48h. Cell cycle was analyzed by flow cytometry. Gene expression and TE activity were assessed by RNA-seq. Differential expression and KEGG enrichment were performed using DESeq2 and clusterProfiler. TE activity was quantified using three complementary pipelines (Telescope, TEtranscripts and ERVmaps) for comparative analysis.

Results: DNMTi and HDACi induced distinct effects on TE regulation and cellular programs. DNMTi triggered S/G2 arrest and repression of proliferation-related pathways (PI3K-Akt, focal adhesion, ECM, FoxO), and led to a predominance of downregulated TEs, mainly LINE-1 elements. In contrast, HDACi did not induce cell cycle arrest and promoted a transcriptionally active state, with upregulation of proliferation-related pathways and an overall increase in TE expression, mainly involving endogenous retrovirus families. Deregulation was consistently higher in the primary cell line compared to the metastatic model.

Comparative analysis across TE quantification pipelines revealed differences in TE detection. Telescope identified a higher number of deregulated elements across conditions compared to TEtranscripts. In contrast, ERVmaps, despite being specifically designed for ERV analysis, detected fewer deregulated elements.

Conclusions: DNMTi and HDACi induce distinct patterns of TE regulation in NSCLC models. Differences across pipelines highlight the impact of methodological choice on TE detection and interpretation.

32. Seeing the unseen metabolites in breast cancer: an untargeted metabolomics approach powered by FT-ICR-MS

Authors and Affiliations

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Abstract

Metabolic reprogramming is a hallmark of cancer that provides insight into tumor biology and may reveal potential biomarkers of disease. However, characterization of the metabolome remains challenging due to the complexity and dynamic range of metabolites in human samples.

In this study, we profiled the metabolome of human breast tumor tissue leveraging the high resolving power of Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in an untargeted metabolomics approach. Samples were obtained from an established breast cancer (BC) patient cohort (GIMM Biobank) at diagnosis, surgery, and metastasis (n=23, in total) and non-tumor controls (n=12), from prophylactic or plastic surgery.

Multivariate analysis revealed distinct metabolic features between tumor and healthy breast tissues, and BC subtype segregation. Univariate analysis within the triple-negative breast cancer (TNBC) subgroup at diagnosis suggested metabolic differences between responders and non-responders to neoadjuvant chemoimmunotherapy, namely the downregulation of sphingosine in non-responders. Through a KEGG workflow, we identified genes linked to enzymatic reactions of the differentially regulated metabolites to further study metabolic determinants of therapy response. Additionally, the putative annotation of a bacterial cell-wall–related metabolite, N-acetylmuramate, highlights potential tumor-microbiota interactions.

Together, these results demonstrate the feasibility of FT-ICR-MS-based untargeted metabolomics for ultra-high-resolution metabolic profiling of BC tissue. This workflow enables the detection of metabolic signatures associated with tumor presence, supports the discovery of biomarkers predictive of therapy response, and may uncover new microbiota interactions. Currently, we are analyzing longitudinal serum samples from the same patients (at diagnosis, on-treatment, surgery, and metastasis) to investigate circulating metabolic biomarkers for BC screening and management.

33. Targeting the Pancreatic Tumor Microenvironment Using Gold Nanoparticle–Based Photothermy

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Abstract

Pancreatic duct adenocarcinoma (PDAC) is a highly lethal malignancy with a 5-year survival of 5–10%, largely driven by a dense, desmoplastic and heterogeneous tumor microenvironment (TME) that promotes tumor progression, immune evasion, and severely limits drug delivery and therapeutic efficacy. As most patients are not eligible for surgical resection, chemotherapy remains the standard of care, although resistance to treatment is frequently observed. Therefore, there is a critical need for advanced three-dimensional (3D) models that accurately recapitulate both the structural organization and complex cellular interactions of the TME. This study aimed to develop a combinatory therapeutic strategy using gold nanoparticle (AuNP)-mediated photothermy to sensitize PDAC spheroids to the FOLFIRINOX chemotherapeutic regimen. AuNP accumulation was assessed by confocal microscopy, while photothermal conversion efficiency was measured using a thermocouple following laser irradiation. The effects on spheroid viability and architecture were evaluated using CellTiter-Glo 3D assays and histological analysis. AuNPs exhibited time-dependent penetration, reaching maximal accumulation at the spheroid core after 24 h (~3-fold higher than at 3 h). Optimized AuNP conditions induced moderate hyperthermia (43.7 ± 0.8 ºC), leading to pronounced disruption of spheroid architecture and weakening of cell–cell interactions.

Histological analysis confirmed reduced cell adhesion, while trypsin-mediated dissociation assays showed complete spheroid disassembly within 25 min, corresponding to a 55% reduction in digestion time. Notably, combination of AuNP-mediated photothermy with FOLFIRINOX further reduced cell viability to ~50%. Overall, this strategy effectively disrupts TME-associated physical barriers, facilitating drug penetration and enhancing therapeutic efficacy in PDAC.

34. Development of a 29-plex CODEX panel for spatial characterization of the breast cancer microenvironment

Authors and Affiliations

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Abstract

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, lacking targeted therapies, with neoadjuvant chemo-immunotherapy (neoCIT) as the current standard of care. While tumor-infiltrating lymphocyte (TIL) abundance predicts pathological complete response (pCR), approximately 40% of TNBC patients fail to achieve pCR, including 15% of those with TIL-rich tumors. Reliable biomarkers for patient stratification according to therapeutic outcome remain a challenge and unmet clinical need. To dissect the mechanisms underlying therapeutic failure, we developed a 29-plex antibody panel targeting epithelial, immune and stromal compartments for high-dimensional spatial profiling of the tumor microenvironment (TME) using CODEX multiplex imaging.

The panel includes markers for tumor cells (PanCK), immune subsets (CD3, CD8, CD68), stromal and vascular compartments (CD31, LYVE-1), and functional states including activation (HLA-DR), exhaustion (TOX), and proliferation (Ki-67), enabling the identification of over 40 distinct cellular populations.

This approach enables spatial characterization of cellular neighborhoods and interactions, including immune-tumor adjacency, immune exclusion, and perivascular enrichment. Preliminary analyses revealed higher tumor-vessel interactions in non-responders, whereas responders displayed immune-dominant vascular interfaces and higher proximity index values, reflecting increased spatial association of $CD8^+$ T cells with blood vessels, suggesting that vascular-associated immune localization may contribute to therapeutic response. In contrast, $CD8^+$ T-cell density and $CD8^+$/tumor cell ratios alone were insufficient to discriminate TNBC responders from non-responders to neoCIT. To capture this spatial complexity, we incorporated an alternation coefficient to quantify the intercalation between immune subsets and tumor cells within tumor nests, providing an additional layer of spatial resolution for patient stratification.

35. Physicochemical and Cellular Evaluation of PAMAM–siRNA Nanocarriers in Triple-Negative Breast Cancer

Authors and Affiliations

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Abstract

Although early detection and treatment of breast cancer have improved substantially, patients with aggressive subtypes, such as triple-negative breast cancer (TNBC), or advanced disease still experience poor outcomes. Gene- and peptide-based therapies, together with nanomedicine, have emerged as promising targeted approaches. In particular, gene therapy enables modulation of gene expression, while polymeric nanoparticles (NPs) enhance delivery by improving stability, biocompatibility, biodegradability, and protection from degradation. In this study, PAMAM-based dendriplexes were developed as carriers for siRNA-mediated gene silencing in breast cancer.

siRNA-loaded PAMAM(G5) dendriplexes were assembled by electrostatic interactions at varying N/P ratios. Loading capacity was evaluated by electrophoretic shift assay. Physicochemical characterization was performed by dynamic light scattering (DLS), including polydispersity index (PdI), zeta potential (ZP), and hydrodynamic diameter (HD), while morphology and size were assessed by transmission electron microscopy (TEM). Cellular internalization was analysed by flow cytometry using Cy3-labelled siRNA in MDA-MB-231 (GFP+/LUC+) TNBC cells.

siRNA loading efficiency reached ~100% even at low N/P ratio. DLS analysis showed PdI values >0.2, indicating moderately heterogeneous populations. Naked PAMAM(G5) was near neutral, whereas dendriplexes exhibited positive surface charge, with ZP increasing proportionally to N/P ratio and HD decreasing with higher charge ratios. TEM revealed irregular aggregates for naked PAMAM(G5), while dendriplexes displayed spherical morphology with an average diameter of 136.5 ± 21.6 nm, consistent with the enhanced permeation and retention range. High cellular uptake was observed, suggesting efficient dendriplex internalization.

Overall, these findings support PAMAM–siRNA dendriplexes as promising nanocarriers for breast cancer therapy, addressing key challenges in delivery efficiency and translational potential.

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36. PAMAM dendriplexes as an efficient platform for siRNA delivery in breast cancer

Authors and Affiliations

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Abstract

Despite advances in early detection and treatment of breast cancer, the prognosis remains poor for a substantial proportion of patients, particularly those with aggressive subtypes and/or advanced-stage disease. Fundamental and translational research have identified several potential therapeutic targets associated with poor prognosis and resistance to therapy. The advent of targeted therapies has transformed the treatment landscape, offering improved efficacy, specificity, and tolerability compared with conventional chemotherapy. In this context, small interfering RNA (siRNA)–based therapy represents a precise molecular strategy that uses double-stranded RNA molecules to silence disease-associated genes. This approach provides high specificity, reversibility, and the potential for sustained therapeutic effects across a range of diseases, including cancer.

In this study, we evaluated the functional properties of dendrimer-based siRNA nanotherapy as a potential platform for gene silencing in breast cancer. The MDA-MB-231 (GFP+/LUC+) triple-negative breast cancer (TNBC) cell line was used as an in vitro model. Dendriplexes were assembled using varying siRNA concentrations, with either unlabeled or Cy5-labelled PAMAM (G5) and a control Cy3-siRNA. Complexation efficiency was determined by indirect fluorescence subtraction. Cytotoxicity of both naked and complexed PAMAM was assessed using the Alamar Blue assay, and siRNA protection was evaluated through an RNase stability assay.

The results demonstrated that complexation efficiency reached 100%, irrespective of siRNA concentration. While naked PAMAM (G5) induced a dose-dependent cytotoxic effect in TNBC cells, PAMAM–siRNA dendriplexes exhibited no detectable cytotoxicity. Furthermore, complexation effectively protected siRNA from enzymatic degradation.

Overall, these findings suggest that this strategy represents a promising platform for targeted gene silencing in breast cancer.

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37. Cytoskeletal imbalances at Hereditary Diffuse Gastric Cancer onset: Filamin A modulates E cadherin-dependent basal extrusion

Authors and Affiliations

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Abstract

E-cadherin germline variants are causative of Hereditary Diffuse Gastric Cancer, a syndrome characterized by the occurrence of diffuse gastric cancer and lobular breast cancer. Patients often present with advanced disease at diagnosis due to its silent and aggressive nature. Interestingly, pagetoid spread of signet-ring cells beneath intact gastric epithelium has been identified as a key precursor lesion of invasive gastric cancer. Evidence suggests that this feature results from a switch from apical to basal extrusion of E-cadherin dysfunctional cells. However, the molecular mechanisms enabling E-cadherin mutant cells to invade the underlying tissue, rather than be eliminated, remain unclear.

To evaluate extrusion phenotypes induced by the loss of E-cadherin, we explored isogenic cell lines expressing either the wild-type protein or mutants affecting distinct protein domains. We established a monolayer system in which labelled mutant cells were surrounded by an unlabeled wild-type context to monitor mutant cell behavior. Cytoskeletal organization, protrusion formation, and cell spreading were further examined in 3D culture, and the expression of cytoskeletal components was modulated to assess their impact on cell delamination.

We demonstrated that cells expressing variants disrupting the juxtamembrane and intracellular regions exhibit increased basal extrusion rates and achieve greater invasion distances, when compared to those expressing extracellular variants. Notably, juxtamembrane and intracellular variants were associated with reduced Filamin A activity and actin remodeling. Depletion of Filamin A in wild-type cells induced a loose cytoskeletal structure, basal nuclear positioning, and integrin upregulation, increasing cell migration into the extracellular matrix.

This work unveils an interplay between E-cadherin and Filamin A that controls epithelial cell extrusion, and provides a rationale for potential therapeutic interventions in the early stages of gastric cancer.

38. Exploring RANK expression heterogeneity effect on breast cancer cell dynamics under therapeutic pressure

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Abstract

Breast cancer (BC) is the most commonly diagnosed cancer and a leading cause of cancer-related death among women worldwide. Although it is highly treatable, survival rates decrease significantly in advanced stages, particularly in metastatic disease. The RANK/RANKL pathway has been implicated in tumorigenesis, as well as in the development of invasiveness, metastasis, and therapeutic resistance.

This study investigates how heterogeneity in RANK expression influences tumour cell behaviour under therapeutic pressure. Isogenic luminal breast cancer cell lines with stable RANK overexpression previously generated and characterized by our group were used to establish heterogeneous co-cultures. For that, MCF-7 parental GFP+ cells were mixed with RANK-overexpressing GFP+ and RFP+ populations at defined proportions. Next, cells were subjected to competition assays under basal conditions and following treatment with ribociclib, fulvestrant, paclitaxel, and doxorubicin, to which RANK-overexpressing cells were found to be resistant. Population dynamics were longitudinally monitored using fluorescence-based measurements.

Preliminary results show an increase in RFP fluorescence over time across all conditions, reflecting the therapy-tolerant behaviour of RANK-overexpressing cells. Moreover, results suggest that the presence of RANK-overexpressing cells within a heterogeneous breast tumor overpowers the presence of RANK-negative/low cells, even at lower frequencies. These findings provide initial evidence that RANK expression modulates breast cancer cell behaviour within heterogeneous populations and support the use of fluorescence-based co-culture systems to study tumour dynamics under therapeutic pressure.

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39. Reprogramming Tumor N-glycosylation to Enhance Innate-like Immunity

Authors and Affiliations

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Abstract

Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with a rising incidence in young adults and limited benefit from current immunotherapies for the vast majority of patients. In mismatch repair-deficient (MMRd) CRC, a major mechanism of immune evasion is HLA class I downregulation, which impairs CD8+ T-cell recognition. In this context, innate-like lymphocytes, including NK cells and γδ T cells, can bypass HLA restriction and provide an alternative anti-tumor immune response. Tumor glycosylation is increasingly recognized as a key modulator of immune recognition, yet its impact on innate-like immune responses in CRC remains poorly understood.

We hypothesize that CRC glycan signatures regulate innate-like lymphocyte function and contribute to tumor immune escape. Our aim is to elucidate how N-glycan remodeling shapes innate-like immune responses and to identify novel glycan-based immunotherapeutic strategies. To this end, we combined in silico analyses of CRC patient datasets, glycoengineered murine CRC models, and coculture assays with innate-like lymphocytes.

In silico, HLA-high tumors displayed increased MGAT5 expression relative to HLA-low tumors, consistent with enrichment of β1,6-branched N-glycan biosynthesis. HLA-high tumors also showed higher inferred scores of innate-like immune populations. Strikingly, MGAT1 expression correlated positively with these innate-like populations, whereas MGAT5 showed no significant correlation, despite being elevated in HLA-high tumors. This suggests that, although HLA-high tumors are enriched in both innate-like immune signatures and MGAT5 expression, β1,6-branched N-glycans are unlikely to be the main drivers of innate-like immune recruitment in this context. Instead, this uncoupling aligns with an immune-shielding role for branched N-glycans in CRC, potentially acting at the level of immune recognition or effector function rather than recruitment.

To functionally test the role of complex branched N-glycans, we cocultured MC38 CRC cells (mock vs glycoengineered) with innate-like lymphocytes from C57BL/6 mice. Glycoengineered MC38 cells showed reduced viability and increased active caspase-3 upon coculture compared with mock cells, indicating enhanced susceptibility to innate-like cytotoxicity. Correspondingly, cocultured innate-like lymphocytes upregulated activation markers CD25, NKG2D, and selected glycan-binding proteins (GBP).

In glycoengineered CRC mouse models, we observed fewer tumors compared with control VCMsh2fl/fl mice. Moreover, we found that tumors from glycoengineered mice showed increased infiltration of innate-like lymphocytes, which presented higher NKG2D and GBP expression. Together, these preliminary data indicate that tumor N-glycan remodeling can shape both the recruitment and functional activation of innate-like lymphocytes in CRC, supporting glycan-targeted strategies to enhance HLA-independent anti-tumor immunity.

40. Macrophages as drivers of resistance to Dabrafenib plus Trametinib therapy in anaplastic thyroid cancer

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Abstract

Introduction: Anaplastic thyroid cancer (ATC) is an aggressive malignancy with poor response to standard therapies. While Dabrafenib plus Trametinib (DT) has improved outcomes in BRAF-mutant ATC, resistance remains a challenge. Macrophages are linked to poor prognosis in ATC, and SPRY4 has emerged as a potential biomarker in ATC–macrophage interactions.

This study aimed to evaluate macrophage-mediated DT resistance and the therapeutic potential of macrophage targeting.

Materials and Methods: We established 2D co-cultures of BRAF-mutant ATC cell lines (T235 and T238) with THP-1-macrophages, and assessed the viability, invasion, ERK1/2, SPRY4, PD-L1 and vimentin expression, and cytoskeletal remodelling, in ATC cells. Macrophage markers (CD68, CD80 and CD163) were analysed in vitro and in tumour samples from eleven DT-treated ATC patients, using flow cytometry, immunocytochemistry and immunohistochemistry.

Results: DT reduced cell viability across all ATC lines, while macrophage co-culture restored it ($p<0.05$). Macrophages increased ATC invasion, with or without DT ($p<0.0001$), evidenced by actin cytoskeletal remodelling and increased vimentin. DT downregulated SPRY4 ($p<0.001$), inhibited MAPK signaling ($p<0.05$), and suppressed PD-L1, and all these effects were reversed by macrophages ($p<0.05$). Under DT, macrophage markers analyses showed M2-like activation in co-culture ($p<0.05$), as well as high infiltration in patients' tumour samples. Targeting macrophages, in combination with DT, significantly enhanced DT effects on viability and invasion and decreased M2-like polarisation ratio ($p<0.05$), bypassing the macrophage-mediated DT resistance.

Conclusions: Our findings show that macrophages modulate ATC response to DT by restoring MAPK signalling, enhancing viability and invasion, and upregulating PD-L1, supporting a pro-tumoural phenotype. Targeting macrophages overcomes this resistance, highlighting the ATC-macrophage axis as a promising therapeutic target to improve DT efficacy.

41. Colorectal Cancer Modeling from 2D to 3D: Uncovering Differential Drug Responses in Colorectal Cancer

Authors and Affiliations

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Abstract

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, largely due to chemoresistance that limits therapeutic efficacy. Traditional two-dimensional (2D) cell culture models fail to capture the structural and biological complexity of tumors, whereas three-dimensional (3D) systems, such as spheroids and patient-derived organoids (PDOs), better preserve tissue architecture and heterogeneity. In this study, we investigated the transition from 2D to 3D models influences drug response in CRC. Doxorubicin-sensitive and -resistant CRC cell lines (cultured in 2D and as 3D spheroids) and PDOs (established from matched normal and tumor tissues) were characterized by immunofluorescence of different biomarkers and by PCR for TP53 and KRAS mutation status. Drug sensitivity was assessed using cell viability assays following treatment with novel metal-based compounds. Significant differences were observed between 2D and 3D spheroid models, highlighting the impact of cellular organization on therapeutic outcomes. PDOs showed patient-specific variability in drug response, reflecting interpatient heterogeneity, while combination treatments with common therapeutic regimens enhanced cytotoxic effects. These findings feature the importance of integrating advanced 3D models to better recapitulate tumor behavior and improve the predictive value of preclinical drug testing in CRC.

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42. Targeting Mutant p53 Reactivation: A Novel Therapeutic Approach for Ovarian Cancer

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Abstract

Introduction: Ovarian cancer (OC) is a leading cause of cancer death among women worldwide, with a poor 5-year survival (~40%). Inactivation of the tumour suppressor p53, largely due to TP53 mutations, occurs in >90% of high-grade serous carcinomas. Beyond tumour suppressor loss, mutant p53 (mutp53) acquires oncogenic gain-of-function properties promoting tumour progression, immune evasion, and therapy resistance. Thus, pharmacological p53 reactivation is a promising strategy. MANIO is a novel small-molecule p53 activator capable of restoring wild-type-like function to mutant p53.

Objectives: This study evaluated MANIO's anticancer potential against OC.

Methods: MANIO effects were evaluated alone or with standard therapies using OC cell lines and patient-derived organoids. In vivo antitumor activity was assessed in a xenograft mouse model harbouring mutp53 through tumour volume and immunohistochemical analyses. Statistical significance was determined using Student's t-test or ANOVA ($p<0.05$).

Results: MANIO exhibited selective antiproliferative activity in OC cells expressing wild-type or mutant p53, whereas p53-deficient cells showed reduced sensitivity. Cell cycle analysis showed G2/M arrest and increased sub-G1, consistent with apoptosis. In patient-derived organoids, MANIO induced dose-dependent growth inhibition, with IC50 values correlating with p53 status. MANIO synergized with chemotherapeutics including the PARP inhibitor olaparib. In vivo, it inhibited tumour growth without toxicity. Immunohistochemistry showed decreased proliferation, increased apoptosis, reduced angiogenesis, and downregulation of epithelial–mesenchymal transition markers.

Conclusion: MANIO demonstrates selective p53-dependent antitumor activity across OC models and enhances standard therapy efficacy, supporting its potential for OC treatment.

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43. An in vitro assay to study endothelial dysfunction in cancer-associated thrombosis towards organ-on-chip development

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Abstract

Cancer-associated thrombosis (CAT) is the second leading cause of death in cancer patients, particularly in gastrointestinal malignancies. Its development is driven by mechanisms described in Virchow's triad, with endothelial dysfunction playing a central role in establishing a pro-thrombotic state. However, current models fail to accurately reproduce tumour–endothelium interactions under physiologically relevant conditions.

An in vitro assay was developed to recapitulate endothelial dysfunction as a key mechanism of CAT and as a step towards organ-on-chip integration. Human umbilical vein endothelial cells (HUVECs) were characterised by RT-qPCR for endothelial markers (ICAM1, NOS3, PECAM1, VWF). A collagen-coated, insert-based permeability assay was performed to assess endothelial barrier integrity. HUVECs were stimulated with tumour necrosis factor alpha (TNF-α) as a positive control, or exposed to conditioned media derived from the gastric cancer cell line AGS.

Stimulation of HUVECs with TNF-α, a pro-inflammatory cytokine known to induce endothelial barrier disruption, resulted in a 1.4-fold increase in permeability compared to untreated controls, consistent with vascular dysfunction in pro-thrombotic states. Confocal microscopy revealed cytoskeletal remodelling with actin reorganisation and elongated intracellular fibres. Exposure of HUVECs to AGS-conditioned media resulted in a dose-dependent decrease in permeability compared to untreated controls, suggesting that tumour-derived soluble factors can modulate endothelial barrier function.

A functional in vitro platform to assess endothelial barrier responses relevant to CAT was established, supporting the transition towards organ-on-chip models. Ongoing work aims to evaluate the effects of additional cancer cell lines using conditioned media and indirect co-culture within the same insert-based system.

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44. Macrophages modulate Radioresistance in TNBC zebrafish xenografts

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Abstract

Triple-negative breast cancer (TNBC) accounts for 10-15% of breast cancers and is associated with poor outcomes and increased resistance to radiotherapy (RT). Mechanisms underlying RT resistance remain unclear, including the role of tumor-associated macrophages (TAMs).

To investigate this, we generated radiosensitive (RS) and radioresistant (RR) zebrafish xenografts from MDA-MB-231 cell lines: the parental MDA-231-RS and its irradiation-derived counterpart MDA-231-RR. Cells were microinjected into 2 days post-fertilization embryos and xenografts were irradiated (25 Gy) at 1 day post-injection (dpi). Tumors were analyzed through confocal microscopy and tumor engraftment, cellular apoptosis, micrometastases formation and TAM recruitment were quantified.

As expected, at 5 dpi, RS tumors showed increased apoptosis upon RT, whereas RR xenografts did not respond. Interestingly, in untreated conditions, RR xenografts engrafted poorly (38%) compared to RS xenografts (73%, $p<0.0001$) but recruited more TAMs (1.7-fold, $p=0.0053$), suggesting increased immunogenicity. RR xenografts also displayed higher metastatic potential (1.7-fold, $p<0.0001$) with distinct dispersion patterns. Furthermore, macrophage depletion with L-Clodronate sensitized RR tumors to RT, suggesting a protective role of TAMs towards RR cells. Upon RT, TNFα- TAMs were selectively reduced, whilst TNFα+ population was unaffected. Given the high expression of PD-L1 in these cells, we also tested atezolizumab, an anti-PD-L1 monoclonal antibody, on RR tumors, and observed a strong anti-tumor effect both as single agent and with RT.

Overall, we characterize the distinct phenotypes of RR and RS TNBC xenografts, revealing a critical involvement of TAMs in RR tumors, and highlight PD-L1 blockade as a strategy to overcome RT resistance.

45. 3D chromatin reorganization profiling during acquired resistance to KRAS inhibition reveals changes consistent with cellular plasticity

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Abstract

Introduction: Despite recent advances enabling mutant-specific KRAS inhibition, clinical responses remain modest, particularly in colorectal cancer. We aimed to characterize the epigenetic mechanisms that enable mutant KRAS CRC cells to rapidly adapt to and tolerate KRAS inhibition.

Materials & Methods: KRAS inhibition was achieved using siRNA or the inhibitor RMC6236 in colorectal cancer cell lines (HCT116, SW480, LS174T). Effects were assessed by spheroid size, cell number, EdU and Annexin V staining, and clonogenic capacity. Chromatin features were analyzed by TEM, PWS, and Hi-C. Protein and gene expression were evaluated by proteomics and RNA sequencing.

Results: KRAS silencing-sensitive cell lines exhibited a significant reduction in cell number and spheroid size, accompanied by changes in cell cycle and apoptosis, supporting their KRAS dependency. Surviving cells entered a quiescent state and displayed differential expression of proteins involved in gene expression regulation, and nucleosome assembly/repositioning. Accordingly, these cells exhibited changes in the 3D physical organization of chromatin, including chromatin compaction, chromatin domain packing scaling, TAD number, and A/B compartment distribution. Additionally, longitudinal RNA sequencing following KRAS silencing revealed higher transcriptional performance. The capacity to exit a quiescent state was dependent on specific chromatin-modifying agents.

Conclusions: Collectively, our findings highlight a potential epigenetic mechanism underlying the rapid adaptation of mutant KRAS colorectal cancer cells to KRAS inhibition, mediated by profound alterations in chromatin organization underlying transcriptomic plasticity, revealing potential avenues for combination therapies to improve treatment outcomes.

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46. PGRMC1 as a Cancer Stem Cell Biomarker associated with Breast Cancer Brain Metastasis

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Abstract

Brain metastases remain a major cause of mortality in breast cancer (BC), with limited therapeutic options and poor prognosis. Cancer stem cells (CSCs) contribute to metastatic dissemination and therapy resistance; yet clinically relevant CSC biomarkers in the brain metastatic setting are scarce.

Using the metastatic BC cell line MDA-MB-231 and its brain-tropic variant, we performed membrane proteomics to identify CSC-associated biomarkers. PGRMC1 was functionally evaluated via pharmacological inhibition (AG-205), using gold standard methods in vitro and in vivo. Functional in vitro validation using CRISPR-Cas9–edited PGRMC1 is currently ongoing. Clinical relevance was assessed across patient cohorts, including metastatic datasets.

PGRMC1 was significantly upregulated in brain-tropic BC cells. Its inhibition significantly reduced the mammosphere-forming capacity in vitro and decreased CSC frequency in the in vivo chick chorioallantoic membrane model, supporting a functional role in CSC maintenance. Clinically, high PGRMC1 expression was correlated with a brain metastasis-associated CSC signature and poorer survival, particularly in estrogen receptor-negative patients. Notably, PGRMC1 was enriched in brain metastases and specifically associated with reduced brain metastasis-free survival.

In conclusion, our data revealed PGRMC1 as a clinically relevant biomarker that drives CSC-like traits and brain tropism in BC, highlighting its potential for patient stratification and targeted therapeutic strategies.

47. Characterization of the microRNA landscape in tissue and plasma-derived extracellular vesicles from testicular germ cell tumor patients

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Abstract

Introduction: Testicular germ cell tumors (TGCT) are the most frequent malignancies in young adult men. A specific microRNA, miR-371a-3p, is entering the clinic for the diagnosis and follow-up of TGCT patients. However, this microRNA is absent from teratoma, highlighting the need for biomarkers to reliably identify this subtype. Extracellular vesicles (EVs) have been studied in the last decade as intercellular communicators and potential disease biomarkers, due to their cargo being protected, including microRNAs. Thus, the main objective of this work is to unveil EV-microRNA profiles in TGCT patient-derived tissue and plasma samples.

Materials and methods: EVs from 10 tumors (5 primary mixed TGCT and 5 metastatic teratomas) and 11 plasma samples (4 primary mixed TGCT, 4 post-chemotherapy (PC) teratoma, and 3 PC necrosis/fibrosis) collected from TGCT patients were isolated through differential ultra-centrifugation and characterized. EV-RNA was then isolated, and small RNA sequencing was performed for EV-derived microRNA profiling, with data analysis being subsequently achieved.

Results: A total of 966 and 875 microRNAs identified for tissue and plasma-derived EVs, respectively. For tumor-derived EVs, 313 differentially expressed (DE) microRNAs were found between mixed TGCTs and teratoma, with pluripotency-related microRNAs at the top of the list, such as the members of the miR-371-373 cluster. Furthermore, top microRNAs' target analysis was also performed, with their respective biological pathways. In plasma-EVs, 67 and 41 microRNAs were found to be DE between mixed GCT vs teratoma and necrosis vs teratoma, respectively.

Conclusions: Altogether, we have unveiled an EV-microRNA primary TGCT/teratoma signature. This pattern has potential to be further enquired in functional and biomarker studies, to shed light on the biology of these tumors and how it can interconnect with potential clinical biomarkers.

48. tubulin PTM signatures and taxane sensitivity in breast cancer models

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Abstract

Introduction: Microtubules are dynamic polymers of α- and β-tubulin diversified by post-translational modifications (PTMs) and isotypes, forming the "tubulin code". This regulatory system is essential for cellular functions and a potential target for cancer chemotherapy. Taxanes like paclitaxel stabilize microtubules to induce mitotic catastrophe, yet resistance severely limits efficacy in breast cancer treatment, particularly in triple-negative and HER2+ subtypes, where taxane-based neoadjuvant therapies are standard.

Materials and Methods: This project will correlate quantitative tubulin PTM profiles assessed by immunohistochemistry and immunofluorescence with phenotypic assays in NCI-60 breast cancer cell lines and patient-derived organoids from IPO Porto biobanks. Causality will be established by perturbing PTM-modifying enzymes using CRISPR-based approaches and pharmacological modulators, followed by functional analysis of proliferation, invasion, migration, chromosomal instability, epithelial-mesenchymal transition markers, paclitaxel dose-response and live-cell imaging of microtubule dynamics.

Results: Recent work from our lab has identified α-tubulin acetylation and detyrosination as key determinants of Taxol sensitivity in vitro, supporting the hypothesis that specific PTM signatures are associated with distinct taxane responses. These findings suggest that specific PTM signatures dictate distinct responses to taxane treatment. Furthermore, our results will confirm that pharmacological or genetic perturbation of the enzymes modulating these PTMs directly influences paclitaxel susceptibility, thereby validating these signatures as predictive biomarkers of treatment efficacy.

Conclusions: This pre-clinical study aims to identify predictive PTM biomarkers to stratify taxane-sensitive and resistant patients, supporting precision oncology, novel combination therapies and personalized treatment strategies for improved clinical outcomes in breast cancer.

49. Outlining the mRNA surveillance threshold: the role of nonsense-mediated decay in CTNNA1-associated hereditary diffuse gastric cancer

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Abstract

Introduction: Hereditary diffuse gastric cancer (HDGC) is linked to germline variants in CDH1 and CTNNA1 genes, encoding E-cadherin and αE-catenin, respectively. While CDH1 transcripts harboring premature termination codons (PTC) undergo nonsense-mediated mRNA decay (NMD) with a defined activity threshold at amino acid (aa) 836, the role and activity threshold of NMD remains understudied for CTNNA1, although theoretical prediction near aa 795. We aim to assess whether and how NMD affects the pathogenicity of truncating CTNNA1 variants to improve variant interpretation.

Methods: Isogenic gastric cancer cells carrying distinct homozygous truncating CTNNA1 variants across the gene locus were generated using CRISPR/Cas9, characterized and validated by sanger sequencing, reverse transcriptase qPCR, flow cytometry, and immunocytochemistry. CTNNA1 mRNA and αE-catenin protein levels were assessed following NMD inhibition through RNAi targeting a key NMD factor. The frequency of 315 clinical phenotypes from CTNNA1 truncating variant carriers within NMD-competent and incompetent regions were analyzed.

Results: A homozygous out-of-frame deletion in exon 11 resulted in a 33- and 5.3-fold decrease in CTNNA1 mRNA and αE-catenin protein levels, respectively, which was reverted by NMD inhibition. HDGC-associated phenotypes were 3-fold more likely to occur before aa 795, in the NMD-competent (n=74; 1 variant per 10.7 aa) than in the NMD incompetent (n=4; 1 variant per 28 aa) region, relative to all other phenotypes. Additional knockout cell lines, already generated and currently under extensive characterization, are expected to further refine the precise NMD boundary for CTNNA1.

Conclusions: Our findings support NMD as a key pathophysiological mechanism in CTNNA1-associated HDGC and suggest that PTC beyond the canonical NMD boundary may reduce HDGC susceptibility. Precisely defining this boundary will be pivotal for improving clinical variant classification and overall patient clinical management.

50. Heparan Sulfate as a Promising Biomarker in Pediatric Osteosarcoma

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Abstract

Introduction: Pediatric sarcomas (PS) are a heterogeneous group of tumors that account for approximately 10% of childhood solid tumors. These include soft tissue sarcomas and bone tumors, with osteosarcoma (OS) being the most common primary bone subtype. Despite therapeutic advances, diagnosis and treatment remain challenging, highlighting the need for novel biomarkers and targeted therapies. Aberrant glycosylation is a feature in cancer and plays a crucial role in several malignant features of cancer cells. Although altered glycans are already used as serological biomarkers in several cancers, their relevance in pediatric OS is still poorly understood. This study aimed to characterize the glycoprofile of pediatric OS and explore their biological role in tumor progression.

Materials and Methods: Using the MG-63 pediatric OS cell line, we screened several glycan epitopes. To infer about the biological role of identified glycan targets, we used CRISPR-Cas9 to knock out target genes involved in glycans the biosynthetic pathway, followed by functional and signaling analyses. Validation was performed in OS patient tissues from Cento Hospitalar Universitário São João.

Results: Heparan sulfate (HS) was identified as the most highly expressed glycan in MG-63 cells relative to osteoblast control cell line. To investigate its function, EXTL3, a critical enzyme in HS biosynthesis, was knocked out. EXTL3 KO cells showed a marked reduction in HS expression, decreased metabolic activity, and increased migratory and invasive capacities. Since HS is known to modulate receptor tyrosine kinase (RTK) signaling, RTK activation was assessed and revealed reduced phosphorylation of c-MET, EGFR, and PDGFRα in KO cells. In patient samples, HS staining was positive in 70% of OS tissues, while absent in adjacent normal bone and cartilage.

Conclusion: Our results identify HS as a promising glycan signature in pediatric OS, highlighting its potential role in oncogenic RTK signaling and its promise as both a biomarker and therapeutic target.

51. 4F-GalNAc reprograms glycosylation-dependent cancer cell traits through sugar donor depletion and enzymatic blockage

Authors and Affiliations

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Abstract

Introduction: Glycosylation is frequently dysregulated in cancer, leading to the expression of tumor-associated glycans, such as Sialyl Lewis X (SLeX), which is involved in tumor progression, invasion, and metastasis. Recently, fluorinated monosaccharide analogs have emerged as promising inhibitors of glycosylation. In this work, we aimed to test novel Fluorinated Galactosamine (F-GalNAc) formulas as inhibitors of cancer-associated O-glycans.

Methods: The inhibitory capacity of the synthesized compounds was assessed by a dose-response assay, and the biological impact of the most promising compound was evaluated both in vitro and in vivo. To determine transcriptional changes, we performed RNA-seq analysis and further validated these changes using mass spectrometry and enzymatic assay of polypeptide N-acetylgalactosaminyltransferase 2 (ppGalNAcT2) activity.

Results: Among the compounds tested, per-O-acetylated 4F-Ac3GalNAc markedly reduced cell-surface expression of O-linked sialyl Lewis X (SLeX) and sialyl Tn (STn), as well as global O-glycosylation. Substitution of acetyl groups with butyryl groups (4F-Bu3GalNAc) enhanced 4F-GalNAc potency by increasing its lipophilicity. Further analysis showed that 4F-GalNAc depleted intracellular sugar donor pools towards the biosynthesis of UDP-4F-GalNAc, and this was not a substrate for polypeptide N-Acetylgalactosaminyltransferase 2 (ppGalNAcT2). Consequently, treated cancer cells exhibited reduced motility and invasion, decreased E-selectin binding, altered receptor tyrosine kinase (RTK) expression and activation, and suppressed tumor growth in vivo.

Conclusions: Together, 4F-GalNAc depletes normal sugar donor biosynthesis, disrupting cancer-associated O-glycan biosynthesis and malignant traits, highlighting its therapeutic potential in glycosylation-driven tumor progression.

52. THOR Methylation signatures in liquid biopsies for early breast cancer detection

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Abstract

Breast Cancer (BC) is the leading cause of cancer-related death among women worldwide. The incidence of BC in young women (<40 years old) is rising, revealing the need to develop and implement new strategies for BC diagnosis beyond current imaging-based approaches. Telomerase reactivation through upregulation of human Telomerase Reverse Transcriptase (hTERT) gene occurs in most BC cases, leading the cancer cells achieve self-renewal capacity. One mechanism responsible for hTERT upregulation is the hypermethylation of a specific region within the hTERT promoter, called TERT Hypermethylated Oncological Region (THOR). Our group showed that THOR is significantly hypermethylated in malignant breast tissue when compared to benign tissue (40.23% vs. 12.81%). Liquid biopsy is a minimally invasive approach that provides real-time information about tumor status. We aim to identify BC-specific THOR methylation signatures for prospective use as liquid biopsy-based biomarkers to detect BC through a blood sample.

To achieve our goal, we established a discovery cohort comprising 59 BC tissue samples, 60 healthy breast tissue samples, and 47 blood samples from healthy individuals. Genomic DNA underwent bisulfite treatment, PCR amplification and Illumina MiSeq sequencing. To identify the BC-specific THOR methylation signatures, we developed a three-step algorithm: read count normalization, Wilcoxon-Mann-Whitney statistical testing ($p<0.05$), and selection of THOR methylation signatures detected in at least 50% of BC samples but absent in at least 80% of healthy samples.

From the discovery cohort analysis, we found that THOR is hypermethylated in BC tissue in comparison with the healthy breast tissue and healthy blood (Kruskal-Wallis $p < 0.0001$). Moreover, we have identified 3 panels of 12, 6, and 10 THOR methylation patterns with high prevalence in BC tissue samples, but rare in the negative controls (specificity > 0.85, sensitivity > 0.80).
Our findings underscore the potential of THOR methylation signatures to be used as sensitive and specific biomarkers for breast cancer detection.

This work is support by the Fundação para a Ciência e Tecnologia (FCT) and Liga Portuguesa Contra o Cancro (LPCC).

53. Deciphering the impact of cancer cell´s secretome and its derived peptide VGF on breast cancer brain metastasis

Authors and Affiliations

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Abstract

Brain metastases represent one of the most serious clinical challenges in breast cancer progression due to their association with reduced survival and limited therapeutic options. In this study, we investigated the impact of the secretome from brain-organotropic breast cancer cells on the establishment of the brain pre-metastatic niche.

Breast cancer cell line 231 and its organotropic variants were used. The secretome was collected from collagen-embedded cultures and analyzed by proteomics. Brain endothelial and microglial cells were incubated with the secretomes and/or derived peptides, and blood–brain barrier integrity and microglial activation were assessed in vitro and in vivo. VGF expression were evaluated using a cohort of primary breast tumors and metastatic samples.

Our findings revealed that the secretome of brain tropic breast cancer cells induces blood–brain barrier disruption and microglial activation in vitro and in vivo. Proteomic analysis identified six dysregulated

peptides, including nerve growth factor–inducible protein VGF. Functional studies demonstrated that VGF compromises blood–brain barrier integrity, alters microglial function, and promotes cancer cell colonization within the brain microenvironment. In patient samples, VGF was detected in tumor and stromal cells and was significantly associated with HER2 overexpression, the triple-negative subtype, and poorer prognosis. Independent validation in an additional cohort, including paired metastases, confirmed the association between VGF expression and increased propensity for brain metastasis, with a significant impact on patient survival.

These findings highlight the role of the secretome of brain-organotropic breast cancer cells in promoting brain pre-metastatic niche formation. VGF emerges as a key mediator of early brain microenvironment remodeling and metastatic colonization, as well as a potential prognostic biomarker and therapeutic target in breast cancer brain metastasis.

54. PARP inhibition alone and combined with CHK1 inhibition or conventional therapies in Acute Myeloblastic Leukemia: an in vitro study

Authors and Affiliations

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Abstract

Genome integrity is essential for cell survival, yet DNA is continuously exposed to insults that activate the DNA damage response (DDR). Dysregulated DDR and increased DNA damage drive genetic instability, contributing to acute myeloblastic leukemia (AML) – a clonal disorder of hematopoietic stem and progenitor cells characterized by impaired myeloid differentiation and uncontrolled proliferation. This study evaluated the therapeutic potential of niraparib (Nir; PARP1/2 inhibitor) alone and combined with CCT245737 (CCT; CHK1 inhibitor) or conventional therapies in AML in vitro.

Seven AML cell lines (HEL, HL-60, K-562, KG-1, LAMA-84, NB-4, THP-1) were used. Cell density and viability were assessed by trypan blue exclusion after Nir treatment (72h). CCT and conventional drug concentrations (cytarabine, imatinib, all-trans retinoic acid) were established in prior studies. Cell death was assessed by flow cytometry (Annexin V/7-AAD) and optical microscopy (May-Grünwald-Giemsa). Combinations were evaluated at 48h. Drug interactions were analyzed using the highest single agent model (SynergyFinder): synergy scores (SC) >10 synergistic, -10 to +10 additive, <-10 antagonistic. $p<0.05$ was considered significant.

Nir monotherapy induced dose- and time-dependent reductions in proliferation and viability, with increased apoptosis. Nir+CCT showed the highest synergy in THP-1 (SC 64; 5μM Nir+5μM CCT; IC50 48h: 53μM) and HEL (SC 47; 5μM Nir+10μM CCT; IC50: 20μM). KG-1 showed antagonism (SC -16; 2.5μM Nir+5μM CCT; IC50: 65μM). With conventional therapies, synergy was detected in THP-1 (SC 33; 10μM Nir+0.1μM cytarabine) and LAMA-84 (SC 24; 7.5μM Nir+0.25μM imatinib; IC50: 65μM); while antagonism was detected in NB-4 (SC -31; 10μM Nir+5μM ATRA; IC50: 19μM).

This study highlights cell line-dependent synergistic effects of combining Nir with CCT or conventional therapies in AML. Dual DDR pathway inhibition showed the most promising outcomes.

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55. Uncovering the long-term immune signature of T-cell lymphoblastic lymphoma

Authors and Affiliations

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Abstract

T-cell lymphoblastic lymphoma (T-LBL) is an aggressive cancer of immature T cells that originates in the thymus. Although treatment advances have improved survival, an increased risk for long-term immune dysfunction in survivors remains a concern. Damage to the thymus from malignant cell expansion or chemotherapy may impair T-cell recovery. Previous studies from our group demonstrated that leukemic T-cell expansion disrupts thymic structure, niche cellular composition, and function.

We hypothesize that T-LBL malignant cells durably damage the thymus, leading to sustained immune dysregulation. We aim to unravel how malignant T cells affect thymopoiesis and how T-LBL regression impacts the thymic microenvironment.

Using ETV6::JAK2 transgenic mice, which spontaneously develop thymic lymphomas, we conditionally expressed the diphtheria toxin (DT) receptor (DTR) in leukemic T cells, enabling their selective ablation upon DT administration. DTR-expressing ETV6::JAK2 mice presenting spontaneous thymic lymphoma (dyspnea and MRI), were treated with DT. Strikingly, treated mice regained a healthy body condition, which persisted for at least 14 days after treatment cessation.

Through flow cytometry detection of typical T-cell surface marker proteins, we found that 14 days after treatment cessation, the minimum time for thymic repopulation, the thymus of mice in T-LBL remission did not fully regain its normal thymocyte cell numbers and population subsets. Furthermore, our preliminary data indicates that after this period, the thymic stroma sustains lasting structural alterations. Ongoing immunofluorescence analysis will assess the status of thymic architecture and key microenvironmental populations.

Our data indicates that 14 days post-remission, thymopoiesis and the thymic stoma remain disrupted. These findings support our hypothesis that T-LBL-induced thymic remodeling leads to durable disruptions in thymopoiesis, potentially contributing to long-term immune defects observed in survivors.

56. Deciphering the Chemo-Sensitizing Potential of Novel Chromene Derivatives in Acute Myeloid Leukemia pre-clinical models

Authors and Affiliations

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Abstract

Acute Myeloid Leukemia (AML) is an aggressive hematologic malignancy characterized by high clonal heterogeneity and limited response to standard chemotherapy, including cytarabine (Ara-C) and anthracyclines. Improving therapeutic sensitivity remains a major clinical challenge.

Epigenetic dysregulation plays a critical role in AML progression and has emerged as a promising therapeutic avenue. This study evaluated novel chromene-derived compounds as chemo-sensitizing agents to enhance the efficacy of standard treatments and to elucidate the underlying molecular mechanisms.

A panel of AML cell lines (THP-1, MOLM-13, KG-1) and HS-5 stromal cells was used to model the bone marrow microenvironment. Cell viability was assessed using resazurin and trypan blue assays. Drug interactions with Ara-C and daunorubicin were analyzed using SynergyFinder 3.0, applying the Bliss independence model to determine synergistic, additive, or antagonistic effects. Mechanistic studies were conducted by Western blot, focusing on apoptosis-related pathways. The most promising combinations were further evaluated in vivo using the chicken embryo model

This work provided a preclinical rationale for combining epigenetic modulators with conventional chemotherapy, potentially contributing to improved therapeutic strategies for AML.

57. ETV6::JAK2 fusion promotes central nervous system invasion in a pre-clinical model of B-cell acute lymphoblastic leukemia

Authors and Affiliations

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy. A subgroup of B-cell ALL (B-ALL) patients with dismal prognosis carries specific genetic alterations, including JAK2 fusions and CDKN2A deletions. Disease relapse in these patients has been associated with central nervous system (CNS) invasion. Here, we hypothesized that JAK2 fusions are a risk factor for development of aggressive CNS-infiltrating B-ALL.

To study the impact of constitutively active JAK2 signaling in CNS involvement by B-ALL, we generated mice expressing an ETV6::JAK2 fusion in a leukemia-prone genetic background (Rag2 and Cdkn2a deficiency).

Indeed, in comparison to Rag2-/-;Cdkn2a-/- littermates, ETV6::JAK2;Rag2-/-;Cdkn2a-/- mice exhibited earlier onset of B-ALL and presented more frequent CNS invasion. In vitro treatment of primary leukemic cells with AZD1480 (JAK2 inhibitor) significantly impaired the survival of ETV6::JAK2-driven B-ALL but not Rag2-/-;Cdkn2a-/- leukemia. Moreover, treatment of wild-type mice infused with ETV6::JAK2;Rag2-/-;Cdkn2a-/- B-ALL cells with JAK1/JAK2 inhibitor Ruxolitinib resulted in a significant reduction of leukemia burden in the peripheral blood, spleen and CNS, in comparison to vehicle group. In parallel, transplantation of both leukemia types in immunodeficient mice revealed that animals injected with ETV6::JAK2;Rag2-/-;Cdkn2a-/- B-ALL presented earlier and significantly higher infiltration of cerebrospinal fluid by leukemic cells compared to those transplanted with Rag2-/-;Cdkn2a-/- leukemia, but similar leukemia burden in the bone marrow. Moreover, the ETV6::JAK2;Rag2-/-;Cdkn2a-/- leukemic cell transcriptome showed higher levels of genes (Selplg and Ccr5) and signaling pathways (unfolded protein response) related to CNS invasion in B-ALL, than that of Rag2-/-;Cdkn2a-/- leukemic cells.

In conclusion, the ETV6::JAK2 fusion promotes leukemia survival, accelerates disease development, and confers increased neurotropism to leukemic cells.

58. The T Cell Glycocalyx in cancer immunotherapy: mechanisms and clinical applications

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Abstract

Immunotherapy has improved cancer treatment; however, its efficacy in colorectal cancer (CRC) remains limited, underscoring the need to uncover new mechanisms of immunoregulation in the patient's immune system and tumor microenvironment (TME). Protein glycosylation is a post-translational modification, and its alteration is considered a hallmark of cancer. In CRC, intratumoral $CD8^+$ T cells showed altered glycosylation, particularly increased β1,6-GlcNAc N-glycans, associated with exhausted phenotypes and higher PD-1 and TIM-3 expression (Azevedo CM et al., Cancer Immunol Res, 2025). The deletion of this suppressive MGAT5-mediated branched N-glycans on therapeutic CD8+ T cells by CRISPR/Cas9, enhances T cell activation and antitumor responses, including in human CAR-T cells. However, the impact of Mgat5 on T cell glycocalyx modulation remains unclear. Thus, this project aims to determine how differential glycogene expression reshapes the T cell glycocalyx in the CRC TME and evaluates glycan signatures as potential biomarkers of immunotherapy response. For this, $CD8^+$ T cells from Mgat5 -/- and wild-type (WT) OT-I mice were isolated, activated, and co-cultured with MC38-OVA, followed by flow cytometry analysis for lectins.

Further, MC38 tumors were established in Mgat5 -/- and WT mice, followed by tumor digestion and flow cytometric analysis of T cell glycocalyx. Preliminary results from in vitro and in vivo experiments show alterations in overall N-glycosylation, mainly in PD-1+CD8+T cells. Strikingly, in vivo, these differences in T cell glycocalyx were observed only in the TME, consistent with decreased tumor growth, but not in the draining lymph (dLN). Our results suggest a different glycan pattern/ arrangement on the surface of mouse CD8+ T cells upon Mgat5 -/-, indicating that Mgat5 contributes to T cell glycocalyx remodeling. PD-1 expression and signals from the TME also play critical roles in shaping the T cell glycocalyx in CRC.

59. Modulation of Tumor Neoantigen Glycosylation as a strategy to improve CD8+ T cell responsiveness in Colorectal Cancer

Authors and Affiliations

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Abstract

Colorectal Cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. This global burden, together with the rising incidence of early-onset cases and the limited efficacy of immunotherapy, underscores the need for more effective immunotherapies. Anti-tumor immune response relies in the recognition of tumor neoantigens presented by Human Leucocyte Antigen (HLA) by CD8+ T cells, triggering their activation/cytotoxic activity. However, in CRC, this process is often inefficient, contributing to immune evasion and tumor progression. Glycosylation, consisting in the post-translational addition of glycans to proteins, has been proven to have a key role in tumor biology, as CRC cells display an overexpression of more complex N-glycans, associated with invasion, metastasis and immune evasion, fostering cancer progression. Nevertheless, how the glycoprofile of CRC HLA-I neoantigen modulates CD8+ T cell activity remains unclear, leading us to hypothesize that glycoengineering of neoantigens may be used as a new therapeutic strategy to enhance CD8+ T cell activity.

We started by analyzing formalin-fixed paraffin-embedded tissue, through PCR, and fresh biopsies of HLA-I CRC human samples, by flow cytometry. Preliminary results show that an increased CD8 expression and cytotoxic activity is associated with an elevated expression of glycogenes linked to the synthesis of less complex N-glycans in CRC samples. Then, by performing an in vitro glycoengineering of a murine colon adenocarcinoma neoantigens toward a less complex N-glycome, in a co-culture setup with CD8+ T cells, we showed these tumor cells expressing a differential/simpler glyco-neopeptidome were more susceptible to T-cell mediated cytotoxicity.

Altogether, these results led us to propose these specific glyco-neopeptidome as strategy to enhance T cell recognition and anti-tumor immune response, paving the way for a new therapeutic avenue in CRC.

60. Mapping the metastatic routes of mutant KRAS cancer cells – the paradigm of colorectal cancer liver metastasis

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Abstract

Introduction: Fifty% of all colorectal cancers (CRCs) will metastasize, and the liver is the preferential colonization site. Resection of colorectal liver metastases (CRLM) associates with a good prognosis, unless a KRAS mutation is present. Indeed, mutant KRAS CRLM exhibit an infiltrative pattern and associate with wider positive surgical margins compared to wild-type tumors. Notably, lung recurrence after curative resection of mutant KRAS CRLM is more common than liver recurrence alone. We aim to evaluate the genomic evolution of mutant KRAS CRC cells along the metastatic process and enlighten if mutant KRAS CRC cells from lung metastasis result from the seeding of liver metastasis upon CRLM resection.

Materials and Methods: Whole-exome sequencing and phylogenetic reconstruction analysis were performed on matched trio FFEP tissues corresponding to stage III primary tumors, resected CRLM, and second lung or liver metastasis of CRC patients from three national hospitals.

Results: The three patients with second lung metastasis (Group A) were all KRAS mutant, whilst the four patients who developed intrahepatic recurrences (Group B) were KRAS wild-type. On average, Group A and B exhibited 310 and 387 single-nucleotide variants (SNVs), respectively, with a greater proportion of nonsynonymous mutations observed in both groups. Phylogenetic analyses revealed that in Group A, the primary tumor and resected CRLM shared a higher number of SNVs, with the lung metastasis being genomically distinct; in Group B, however, the resected CRLM and second liver metastases shared more SNVs.

Conclusions: This study suggests branch-off and sequential migration models for mutant and wild-type KRAS CRCs, respectively. Thus, second mutant KRAS lung metastases likely do not arise from CRLM, but rather may be triggered by signals activated upon their resection.

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61. Extracellular matrix composition dictates adaptive responses of E-cadherin-deficient cells

Authors and Affiliations

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Abstract

E-cadherin is a key regulator of epithelial integrity, and its loss is strongly associated with invasion and metastasis. However, the mechanisms by which loss of cell-cell adhesion promotes cancer cell survival and dissemination remain unclear. We propose that cancer cells exploit dormancy as a survival strategy, by adapting to restrictive extracellular matrix (ECM) conditions that disrupt differentiation programs during tumour progression. Our main aim is to identify an ECM-induced dormancy signature that may uncover novel therapeutic vulnerabilities in cancer cells.

Methods: We established an in vitro model of cell lines transfected with vectors encoding wild-type E-cadherin or the I326N and E758G variants identified in diffuse gastric cancer patients. E-cadherin expression and localization were assessed by Western blot and immunofluorescence. Cells were grown as 3D spheroids embedded in distinct ECM conditions, which were then subjected to structural organization analysis and proteomic profiling by high-resolution mass spectrometry (LC-MS).

Results and Discussion: The I326N and E758G variants showed reduced total protein levels and membrane localization, when compared with wild-type E-cadherin, consistent with functional impairment. In 3D cultures, wild-type cells formed larger and compact aggregates, whereas mutant cells generated smaller spheroids that dispersed more diffusely throughout the ECM scaffold. Proteomic analysis of cellular profiles revealed that the ECM can modulate the molecular state of E-cadherin mutants, with heterogeneous matrices inducing distinct signatures compared to collagen I or matrix-free conditions.

Conclusions: This work provides evidence that ECM composition critically influences the structural and proteomic landscape of E-cadherin-deficient cells, supporting adaptive responses and unveiling associated readouts as potential targets for therapeutic intervention.

62. NK cell adoptive transfer suppresses metastasis and prolongs survival in pancreatic cancer

Authors and Affiliations

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd leading cause of cancer-related deaths, with metastatic disease accounting for ~90% of patient mortality. Yet, effective therapies to limit dissemination remain lacking.

Here, we identify NK cells as key regulators of metastatic competence in PDAC. Using genetic and antibody-mediated depletion models, we show that NK cell loss significantly increases liver and lung metastatic burden and GFP-tagged circulating tumor cells (CTCs) counts.

Mechanistically, NK cell depletion modulated myeloid lineage function, with a shift toward stromal localization, potentially enhancing their capacity to aid invasion and intravasation. Simultaneously, NK cell loss induced activation of epithelial-mesenchymal transition (EMT), proliferation and migration-associated transcriptional programs in cancer cells. Supporting the clinical relevance of these findings, human basal PDAC tumors – characterized by increased metastatic fitness – exhibit reduced NK cell infiltration.

Therapeutically, adoptive NK cell transfer reversed these phenotypes across multiple PDAC mouse models, reducing CTC burden, suppressing metastatic outgrowth, and prolonging survival. In patient-derived xenograft (PDX) models, systemic NK-92 cell therapy reduced metastatic recurrence following tumor resection. Moreover, NK-92 cells demonstrated robust cytotoxicity against patient-derived PDAC organoids. Concordantly, human tumors enriched for activated or activation-prone NK cells were associated with better survival.

Collectively, these findings redefine NK cells as key regulators of metastatic competence in PDAC, by limiting intravasation, CTC survival, metastatic seeding and colonization, while restraining tumor and microenvironmental reprogramming toward pro-invasive states. Notably, these results provide a mechanistic and translational rationale for the development of NK-based immunotherapeutic strategies aimed at preventing and treating metastatic progression.

63. Pathologies localizing and therapy accessing guide for precision navigation

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Abstract

The current work refers to an innovative device that produces data in imaging technics, such as C.T. scan, M.R.I. and even X rays, in order to localize pathologies in the human body and guide accession to them with accuracy, simplicity and low cost. The Guide, as we name it, owns an international patent and is has been tested clinically in patients for proof of its accuracy.

Internationally, the dynamics of an invention of this technology sector are placed in the context of everyday use in diagnostic and invasive, both in the western and emerging economies. The current work refers to an innovative device that produces data in imaging technics, such as C.T. scan, M.R.I. and even X rays, in order to localize pathologies in the human body and guide accession to them with accuracy, simplicity and low cost.

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64. Modulating the RNA-binding protein MEX3A to enhance chemosensitivity in colorectal cancer tumoroid models

Authors and Affiliations

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Abstract

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide, highlighting the need for new therapeutic strategies. RNA-binding proteins (RBPs) regulate RNA processing and are frequently altered in cancer. The stem cell-specific RBP MEX3A is overexpressed in 85% of human CRC cases and controls the stem cell/differentiation balance. Our data suggests that CRC tumoroids lacking MEX3A exhibit increased chemosensitivity. However, the full potential of MEX3A as a therapeutic target remains unexplored.

Material and methods: Patient-derived CRC tumoroids (PDCTs) with MEX3A overexpression were identified by western blot. CRISPR/Cas9 was used to abrogate MEX3A in PDCTs. KO and parental lines are being compared regarding growth, self-renewal and differentiation. For in vivo studies, parental PDCTs were implanted subcutaneously in nude mice to establish xenograft models. Tumour growth curves are being monitored to characterize the models.

Results: We identified high MEX3A expression in five PDCT lines, which were selected for CRISPR/Cas9 editing. Cas9/sgRNA was delivered by electroporation and monoclonal populations obtained by GFP-positive single-cell sorting. Successful KOs were confirmed in five PDCT lines. Preliminary observations indicate differences in tumoroid size and morphology between KO and parental lines, suggesting an impact of MEX3A loss on growth and differentiation. Growth curves generated from the five parental PDCT xenografts in nude mice showed successful tumour development in vivo. These preliminary data provide a foundation for future studies evaluating MEX3A modulation in vivo and treatment response.

Conclusions: Our physiologically relevant ex vivo and in vivo study models hold significant promise for establishing MEX3A as a viable therapeutic target for future drug design.

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65. Molecular profiling of cutaneous melanoma: implications for prognosis assessment and therapy response prediction

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Abstract

Melanoma is a malignant tumor that arises from the transformation of melanocytes. In 2022, it was the 17th cancer with higher incident and the 22nd more deadly. The MAPK pathway regulates cell proliferation and survival, and is often dysregulated in melanoma, mainly through mutations in BRAF or NRAS. Mutations in TERT promoter (TERTp) are also common, potentiating cell immortalization. Although BRAF status is an established biomarker for target therapy response, the role of molecular alterations in prognosis determination and remaining therapy response in melanoma is still not consensual. This study aims to improve melanoma prognosis determination and therapy response prediction, guided by the molecular profile of the tumors.

The current cohort is composed of 294 FFPE melanoma samples from Capuchos Hospital (EC INV195), IPO-Lisbon (EC UIC/1504) and Santarém Hospital (EC HDS134A.05). The samples were subjected to histopathological evaluation, DNA extraction, PCR amplification and Sanger Sequencing to identify hotspot mutations in BRAF, NRAS and TERTp. Statistical analysis is being performed to study the correlation between the molecular profile and clinicopathological and therapy response data.

In the analyzed cohort, 63% of the cases are superficial spreading subtype, and 49% were diagnosed at >67 years (median age). Hotspot mutations in BRAF, NRAS and TERTp were found in 37%, 15% and 38% of the cases, respectively. In hotspot-mutated cases, a statistically significant correlation and risk of presenting

clinicopathological features linked to poorer prognosis was found. The presence of hotspot mutations was also associated with shorter relapse free time. Co-occurrence of TERTp and BRAF mutations seems to define a significantly more aggressive clinical phenotype.

This study offers insights into the significance of molecular profiling in predicting patient's prognosis and therapy response and can support further investigations for prognosis and predictive biomarkers determination.

66. Development and Validation of Genetic Tools to Monitor Endosomal Recycling Dynamics in Invasive Triple-Negative Breast Cancer

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Abstract

Introduction: Triple-negative breast cancer (TNBC) is a major clinical challenge due to its heterogeneity, high metastatic relapse, and lack of targeted therapies. FER kinase promotes TNBC progression by regulating endosomal recycling (ESR), a process essential for cell invasion. FER depletion leads to accumulation of Rab11-positive slow recycling vesicles and reduced Rab4-dependent fast recycling. SEC16A was identified as a potential FER downstream effector, with in silico analysis predicting Tyrosine 1320 (Y1320) as an interaction site. This study aims to characterize the FER–SEC16A interaction and develop fluorescent tools to monitor ESR dynamics in real time.

Materials and methods: shRNA-resistant SEC16A constructs were generated: phosphomutant (Y1320A) and phosphomimetic (Y1320D). These were expressed in SEC16A-depleted MDA-MB-231 cells to assess the number of Rab4 vesicles and Paxillin-rich focal adhesions (FA), by immunofluorescence. Lentiviral constructs were developed to generate Rab4-mCherry and Rab11-GFP tagged MM231 cells (parental and FER iKD), and patient-derived organoids. Validation included Western blot, cell cycle analysis, and live/fixed imaging.

Results: SEC16A depletion reduced Rab4 vesicles and FA. Y1320A failed to rescue these defects, whereas Y1320D restored both to wild-type levels. Fluorescent tools were correctly expressed, exhibited colocalization with endogenous markers, and did not interfere with cell proliferation. Rab4-mCherry recapitulated endogenous behavior, showing reduced vesicle number upon FER knockdown. Live imaging further revealed decreased Rab4 trafficking speed.

Conclusions: Phosphomimetic rescue demonstrates that SEC16A Y1320 phosphorylation acts as a molecular switch regulating ESR and adhesion dynamics. These findings suggest that FER interaction with phospho-SEC16A coordinates membrane trafficking and invasion. The validated dual-reporter system provides a robust tool to study these membrane trafficking dynamics in real time.

67. Exploring CD147/BSG -associated metabolic and immune features across MSI and MSS colorectal cancer

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Abstract

Colorectal cancer (CRC) response to immunotherapy is largely restricted to microsatellite instability-high (MSI-H) tumors, characterized by high tumor mutational burden and neoantigen load, whereas most CRCs are microsatellite stable (MSS) and remain refractory. While this is often attributed to differences in immunogenicity, tumor-intrinsic programs, including metabolic adaptation and microenvironmental conditioning, may also shape immune behavior across CRC subtypes. Tumor metabolism has emerged as a key regulator of the tumor-immune interface, influencing immune cell recruitment, activation, and function. In this context, CD147 (BSG), a multifunctional transmembrane glycoprotein, acts as a chaperone for monocarboxylate transporters (MCT1/MCT4), facilitating lactate export and contributing to a metabolically hostile tumor microenvironment. Beyond its roles in tumor progression and metabolic rewiring, CD147 has been implicated in tumor-immune crosstalk through metabolic competition and immune regulation. However, how CD147-associated tumor programs relate to immune features across CRC contexts, particularly between MSI and MSS tumors, remains unclear. Here, we aimed to explore the molecular and immune landscape associated with CD147 expression in CRC across MSI and MSS subtypes. We performed an exploratory in silico analysis using TCGA COAD/READ datasets. BSG expression was evaluated in tumor versus normal tissues and according to MSI status. Correlation analysis, pathway enrichment and immune deconvolution were used to explore metabolic, hypoxia, antigen-presentation and immune-regulatory signatures, complemented by single-cell RNA-seq analysis to assess cellular localization.

BSG was increased in CRC tumors and showed modest enrichment in MSI tumors, while remaining broadly expressed across subtypes. Higher BSG expression associated with hypoxia, tumor mutational burden and MSI score. Transcriptomic analyses linked BSG-high tumors to cell-cycle progression, mitochondrial metabolism and glycolysis-related programs. BSG also correlated with antigen-presentation genes and LAG3, whereas immune deconvolution showed weak and inconsistent associations with lymphocyte infiltration. Stratified analyses suggested that BSG does not clearly separate immune markers within MSI tumors, where immune activation is already prominent, but may capture selected immune and metabolic features in MSS tumors. Single-cell data indicated that BSG is mainly expressed by epithelial/malignant cells, with low expression in immune populations.

Overall, CD147/BSG is associated with a tumor-intrinsic, metabolically active phenotype that intersects with immune-related features in CRC. Rather than a simple checkpoint marker, CD147 may reflect a context-dependent tumor program with relevance for immune modulation, particularly in MSS disease.

68. A 3D tumour-vessel-on-a-chip microfluidic platform to study breast cancer intravasation and vascular toxicity

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Abstract

Metastasis remains the predominant cause of mortality in breast cancer (BC) patients. Intravasation - the process by which cancer cells enter circulation, represents a critical yet poorly understood event. Elucidating the mechanisms governing this process is essential to reduce or prevent metastasis. During intravasation, cancer cells migrate through and invade the surrounding extracellular matrix (ECM) towards blood vessels, and subsequently transmigrate across endothelial monolayers under fluid shear stress. We have recently developed a tumour-vessel-on-a-chip to study cancer cell invasion and intravasation. The device is composed of five independent microchannels and allows testing BC cell preferential migration. BC cells displayed 1.61-fold higher invasion towards a channel filled with complete cell culture media compared to an ECM-filled channel, suggesting higher migration towards a nutrient source. Upon perfusion, we were able to recover intravasated BC cells. Preliminary morphological analysis of these cells suggests they have reduced cell area and increased circularity (circularity index: control cells = 0.312, intravasated cells = 0.348±0.03) compared to non-intravasated controls. Furthermore, dactolisib, a PI3K/mTOR inhibitor, reduced intravasation in the chip, however, vascular disruption was not assessed yet can be crucial for drug-safety testing. The system also enables the assessment of endothelial barrier integrity based on VE-cadherin immunofluorescence. Using thrombin as a positive control we have optimized an image analysis pipeline which allowed to detect that dactolisib increases endothelial disruption (disruption area: control = 0.017 %, dactolisib = 3.604 %, thrombin = 8.374 %). Overall, this platform constitutes a robust model for advancing the mechanistic understanding of intravasation and for supporting the development of therapeutic interventions, while enabling integrated assessment of vascular safety.

69. Circulating lncRNA MALAT1 in Tumor-Educated Platelets: A liquid biopsy approach for multiple myeloma patients

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Abstract

Multiple myeloma (MM) is a genetically complex clonal plasma cell disorder with high biological and clinical heterogeneity. MM diagnosis and monitoring rely on bone marrow aspirates, invasive procedures unable to capture its clinical/spatial heterogeneity. Tumor-educated platelets (TEPs) have emerged as a less invasive liquid biopsy for diagnosis and real-time monitoring of various tumor types. Long non-coding RNAs (lncRNAs) have been shown to play critical roles in tumorigenesis and tumor progression, with several lncRNAs reported to be aberrantly expressed in MM. This study aimed to identify lncRNAs as non-invasive platelet biomarkers in MM. Platelet RNA-seq data (GSE183635) from 22 MM patients at diagnosis and 183 healthy donors (HD) were used to identify DEGs. Bioinformatic analyses used R/RStudio. qPCR validated lncRNAs in a cohort of MM patients at different disease stages. A total 81 MM patients [37F/44M; 68 years (43-83)] and 47 HD [23F/24M; 58 years (33-95)] were included. $P<0.05$ was considered statistically significant. 223 DEGs were identified between MM TEPs and HD [184 up-regulated (fold change>2) and 39 down-regulated (FC<-1.5); $p<0.001$]. Amongst the DEGs, lncRNA MALAT1 showed promising results (FC=2.128; p=3.4x10-11). In our cohort, at diagnosis MM patients showed higher MALAT1 levels [n=5; med:11.9; IQR:0.6-19.4] vs HD (n=47; med:0.8; IQR:0.2-2.7; p=0.03), supporting it as a diagnostic biomarker (AUC:0.81; CI95%:0.58-1.00; cut-off:9.3; sens:100%; spec:60%; p=0.007).

In remission/response post-transplant, lower values were observed (med:0.07; IQR:0.03-0.14; p=0.04 vs diagnosis; p=0.02 vs progression), suggesting this lncRNA as a response biomarker. At progression, MALAT1 levels are higher (med: 0.84; IQR: 0.23-2.71) than at remission, but lower than at diagnosis. Our results provide proof of concept that MALAT1 expression in TEPs may be a promising non-invasive biomarker for MM patient diagnosis and monitoring. Longitudinal studies in larger cohorts are needed for clinical validation.

70. Bridging Imaging and Therapy: The Role of [64Cu]CuCl2 in Patient-Derived Glioblastoma Models

Authors and Affiliations

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Abstract

Introduction: Targeted radionuclide therapy allows specific irradiation of localized and disseminated disease with fewer side effects than external beam radiation therapy. Glioblastoma (GBM) is the most lethal primary brain tumor; despite surgery, chemotherapy, and EBRT, recurrence is nearly universal due to tumor aggressiveness and heterogeneity. We previously showed that [64Cu]CuCl2 significantly reduces growth and viability in GBM cell line-derived spheroids [1]. Now we evaluate the translational potential of [64Cu]CuCl2 in advanced 3D patient-derived models (PDM) of mesenchymal and proneural GBM subtypes.

Materials and Methods: PDMs used are glioma stem-like cells of mesenchymal and proneural origin [2]. We studied [64Cu]CuCl2 cytotoxic effects, anti-proliferative capacity, and uptake in 3D cultures (mimicking microregions) and isolated cells (mimicking tumor-initiating cells). Biodistribution studies were performed in athymic nude mice bearing GBM xenogfratfs.

Results: Proneural cells (GSC23, GSC7-11) were the most sensitive to 64Cu, showing low viability and proliferation despite lower uptake in GSC7-11 spheres. Mesenchymal cells exhibited the highest uptake but greater resistance. Additionally, isolated cells were more susceptible to radiation treatment than 3D cultures. Biodistribution confirmed 64Cu accumulation and retention in both tumor subtypes, with primary hepatobiliary excretion.

Conclusions: This work validates the potential of [64Cu]CuCl2 as a theranostic radiopharmaceutical suitable for different subtypes of glioblastoma, being particularly effective in proneural GBM tumors. Furthermore, GBM PDMs were benchmarked for the translational evaluation of new target-specific radiopharmaceuticals in a personalized medicine approach.

References

1 - Pinto, CIG, et al. (2024). EJNMMI Research, 14(26).

2 - Bhat, KPL, et al. (2013). Cancer Cell, 24(3), 331-346.

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71. Uncovering the mediators of PSGL-1 glycoprotein immune checkpoint function in non-Hodgkin B-cell lymphoma

Authors and Affiliations

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Abstract

Non-Hodgkin B-cell lymphomas are a heterogeneous group of hematologic malignancies driven by clonal B-cell proliferation. Despite advances in immunotherapy and CAR-T therapy, most non-Hodgkin lymphomas remain unresponsive to immune checkpoint inhibitors, highlighting the need for novel immunomodulatory strategies. P-selectin glycoprotein ligand-1 (PSGL-1) has emerged as a potential immune checkpoint that negatively regulates T-cell function and promotes exhaustion in murine cancer models. Our group demonstrated that PSGL-1 blockade in immunocompetent mouse models with B-cell lymphoma results in decreased tumor progression as well as the recruitment and activation of T cells. Here, we aimed to identify immune cell populations and ligands that mediate the PSGL-1 immunoregulatory function in B-cell lymphoma.

We found that PSGL-1 expression was detected across multiple immune populations within the lymphoma microenvironment, both in murine models and in diffuse large B-cell lymphoma (DLBCL) patients. Importantly, PSGL-1 blockade failed to control tumor progression in T cell-deficient mice and was similarly impaired upon macrophage depletion. These findings indicate that T cells and macrophages are key mediators of the anti-PSGL-1 therapeutic effect and reinforce the role of PSGL-1 in modulating the immune response in B-cell lymphomas.

To evaluate the expression of PSGL-1 ligands, we analyzed scRNA-seq datasets from four DLBCL patients. We observed a strong correlation between SELPLG (which encodes PSGL-1) and VSIR (which encodes VISTA) expression. VISTA has recently been identified as a PSGL-1 ligand in acidic tumor environments. Consistently, SELPLG expression was enriched in T cells, whereas VSIR was predominantly expressed in macrophages, pointing to a potential role for PSGL-1/VISTA interactions in immunosuppression. Notably, VISTA blockade likewise resulted in delayed mouse tumor progression, supporting the development of novel immunotherapeutic strategies targeting the PSGL-1/VISTA axis.

72. Dual-imaging radiocomplex targeting PSMA for prostate cancer theranostics

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Abstract

Introduction: Prostate cancer (PCa) remains one of the leading causes of cancer-related death in men. Diagnosis often starts with prostate-specific antigen (PSA) testing and clinical assessment, with prostate-specific membrane antigen (PSMA) being considered the best-established target antigen in PCa, due to its high expression in prostate tumor cells, at all tumor stages. Although PSMA-targeted Positron Emission Tomography (PET) tracers such as ^{68}Ga-PSMA-11 (locametz) have been a staple in clinical application for PCa, these agents do not provide real-time optical guidance during surgery. In this regard, we propose a PSMA-targeted dual-imaging radiocomplex that integrates ^{68}Ga and a near-infrared fluorescence probe indocyanine green (ICG) in the same molecular structure, capable of both preoperative PET imaging and intraoperative image-guided surgery

Materials and Methods: The dual-imaging compound (ICG-DOTA-PSMA) was synthesized and characterized by ESI-MS and HPLC. Current work involves ^{68}Ga radiolabelling of the conjugate using an automated synthesis module, used in clinical practice. Cellular uptake studies will be performed in PSMA-positive (LNCaP) and PSMA-negative (PC3) prostate cancer cell lines, and fluorescence microscopy will assess dye's localisation and accumulation.

Results: We successfully synthesized the desired precursor compound (ICG-DOTA-PSMA) in high purity. The construct contains a Glu-urea-Lys motif for PSMA targeting, a Phe-TXA-Lys linker, a DOTA chelator for ^{68}Ga coordination, and ICG. Current work focuses on optimising ^{68}Ga radiolabelling and setting up PSMA-positive (LNCaP) and PSMA-negative (PC3) cell assays to assess uptake and specificity.

Conclusions: The dual-imaging platform ICG-DOTA-PSMA was successfully synthesized. The compound is expected to provide a stable coordination to ^{68}Ga, and suitable cellular uptake in PSMA-positive PCa cell lines, demonstrating its potential for preoperative PET imaging and intraoperative image-guided assistance.

73. MRP8 Drives Inflammation-Dependent Immune Reprogramming and Th17 Polarization in Pancreatic Ductal Adenocarcinoma

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Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) develops within a chronically inflamed microenvironment that fuels tumor progression and early metastasis. Inflammatory signaling drives immune cell recruitment into the tumor microenvironment and reprograms infiltrating immune populations toward immunosuppressive and tumor-promoting phenotypes. However, the mediators linking inflammation to immune remodeling in PDAC remain poorly defined. MRP8/S100A8, a myeloid-derived protein, forms the calprotectin heterodimer with MRP14/S100A9. Although MRP8 signaling is well established in inflammatory diseases, its role in PDAC progression and immune remodeling remains largely unexplored.

Material and Methods: Acute and chronic pancreatitis were induced by caerulein in mice. A constitutive S100A8 knockout (KO) mouse was crossed with Pdx1-Cre and KrasG12D alleles to generate KCS100A8KO mice. Further crossing with Trp53R172H generated KPCS100A8KO mice; KPC mice used as control. MRP8 expression and immune infiltration were assessed across GEMMs, orthotopic PDAC models, and human PDAC samples.

MRP8 Immunohistochemistry was performed on patient PDAC tissues, and associations with clinicopathological features and survival were evaluated.

Results: MRP8+ cells progressively accumulated during PDAC development, from early pancreatic lesions to advanced tumors, across all models analyzed. Increased MRP8$^+$ cell infiltration correlated with fibrosis, immune infiltration, and activation of inflammatory pathways. In pancreatitis models, MRP8$^+$ cells were markedly enriched in the pancreas, supporting a role for MRP8 in inflammation-driven immune recruitment. Clinically, high MRP8$^+$ cell infiltration was associated with reduced patient survival, underscoring its biological and prognostic relevance.

Conclusion: MRP8 appears to connect pancreatic inflammation with immune remodeling in PDAC, promoting a tumor-supportive microenvironment and representing a potential biomarker and therapeutic target.

74. Establishment and characterization of a colorectal cancer model of acquired resistance to KRAS inhibition

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Abstract

Introduction: Mutations in KRAS are highly prevalent in colorectal cancer (~40%), yet responses to KRAS-targeted therapies remain limited due to rapid resistance. Previous work from our group showed that KRAS silencing induces chromatin reorganization and cellular plasticity. Herein, we established and characterized a model of acquired resistance to KRAS inhibition and investigated whether targeting chromatin dynamics can improve therapeutic response.

Material and Methods: Resistance to the KRAS inhibitor RMC6236 was induced in HCT116 through gradual exposure to increasing concentrations of the drug (100 nM to 12.5 μM), generating independent resistant populations (RMC1 and RMC2). Responses were evaluated through population doubling, apoptosis analysis, and chromatin compaction by TEM. The response to chromatin-remodeling agents was assessed using clonogenic assays.

Results: Despite elevated basal apoptosis, RMC1 and RMC2 populations exhibited robust resistance to KRAS inhibition, maintaining proliferative capacity and survival under 12.5 μM RMC6236, whereas control cells showed marked induction of apoptosis and growth arrest upon exposure to the inhibitor. Chromatin analysis confirmed that KRAS inhibition decreases chromatin compaction; however, the reduction is less pronounced in 12.5 μM RMC1- and RMC2-resistant populations than in control cells treated with the inhibitor. Clonogenic assays revealed that resistant populations remain sensitive to chromatin-remodeling agents, suggesting a persistent vulnerability despite acquired resistance.

Conclusion: We have established a cell CRC cell model resistant to the KRAS inhibitor RM6236, revealing it as a useful model to study the role of chromatin remodeling as a mediator of resistance to KRAS inhibition and as a target to restore sensitivity.

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75. Defining and Enhancing NK Cell Control of Metastasis in Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with limited therapeutic options. We previously demonstrated that natural killer (NK) cells are key regulators of PDAC progression, yet their therapeutic potential remains underexplored.

Here, we show that NK-92 cells infiltrate patient-derived organoids, induce apoptosis, and suppress tumor growth. Proteomic analyses revealed activation of NK cytotoxic programs, while PDAC cells upregulated stress-response and immune-evasion pathways. Our lab has demonstrated that adoptive transfer of NK-92 cells reduced metastatic burden in patient-derived xenograft (PDX) models, limiting dissemination and decreasing postoperative recurrence. Together, these findings support NK-92 cells as a promising therapeutic platform for PDAC, although improved persistence, resistance to the tumor microenvironment, and tumor-specific recognition are needed.

To address these limitations, we developed a strategy to improve NK cell-based immunotherapy in PDAC by combining tumor targeting with reinforcement of intrinsic NK function. Candidate tumor-associated targets were identified through proteomic profiling of patient-derived organoids to support CAR-NK recognition and selective elimination of PDAC cells. Target expression is being validated by immunohistochemistry in PDX tissues, and confirmed targets will be tested in PDX-derived cancer cells using siRNA-mediated inhibition. In parallel, intrinsic NK-enhancing factors are being examined by assessing basal expression through immunofluorescence, followed by targeted modulation in NK-92 cells to evaluate their impact on cytotoxicity and persistence. Engineered NK-92 cells will be co-cultured with patient-derived organoids to test whether these changes improve NK activity using flow cytometry and cytokine profiling as readouts.

Overall, this work provides a translational framework for next-generation CAR-NK strategies and optimized NK cell therapies in PDAC.

76. IL-7R signaling drives lipid metabolic reprogramming in acute lymphoblastic leukemia

Authors and Affiliations

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Abstract

Introduction: Acute lymphoblastic leukemia (ALL), the most common childhood malignancy, arises from clonal expansion of B- or T-lymphoid progenitors blocked during development. Interleukin-7 (IL-7) and its receptor (IL-7R) are critical for normal lymphopoiesis but can also drive leukemia progression and CNS infiltration. Given that metabolic reprogramming is a cancer hallmark, we investigated whether IL-7 rewires lipid metabolism in ALL.

Materials and Methods: Quantitative proteomic and transcriptomic analyses were performed to identify novel IL-7R signaling mediators. Lipid and cholesterol accumulation were measured alongside metabolic gene expression and key transcription factor levels. Cell cycle progression and viability following pharmacological inhibition of lipid metabolism enzymes were assessed by flow cytometry.

Results: IL-7 stimulated a transcriptional program favoring lipid anabolism, upregulating genes involved in fatty acid and cholesterol biosynthesis. This response was validated by qRT-PCR and was dependent on IL-7–mediated PI3K/AKT/mTOR signaling. IL-7 also increased precursor and nuclear forms of a master lipid-synthesis transcription factor and promoted intracellular lipid and cholesterol accumulation. Pharmacological blockade of lipid metabolism reduced leukemic cell viability and impaired cell cycle progression; IL-7 partially rescued these effects, demonstrating that IL-7–driven metabolic rewiring sustains leukemic proliferation and survival.

Conclusions: IL-7 induces lipid metabolic reprogramming via mTOR-dependent anabolic pathways, supporting ALL maintenance. These findings highlight lipid metabolism as a novel therapeutic vulnerability in IL-7R-dependent ALL.

Funding: Gilead Grants Program, FCT, LPCC-NRS, "la Caixa" Foundation, Worldwide Cancer Research

77. Development of anti-IL-7Rα antibodies as a targeted therapy for T-cell Acute Lymphoblastic Leukemia

Authors and Affiliations

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Abstract

Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive blood cancer. Despite high therapeutic efficacy in children, adult and refractory/relapse cases remain a significant hurdle. Interleukin-7 receptor (IL-7R) is expressed in around 70% of T-ALL cases and IL-7 promotes T-ALL cell proliferation and viability. Moreover, 10% of T-ALL cases display IL-7Rα gain-of-function mutations. T-ALL cell dependence on IL-7Rα provides a robust rationale to target this pathway using anti-IL-7Rα monoclonal Antibodies (mAbs).

Materials and Methods: To identify anti-human IL-7Rα mAbs with clinical potential, we evaluated the ability and specificity of a panel of 48 mAbs (generated by FairJourney Biologics) to bind to human IL-7Rα, using flow cytometry (FC). mAbs with high binding capacity were further characterized for their: 1) ability to block IL-7-mediated signaling in T-ALL cells (western blot); 2) impact on the viability and proliferation of T-ALL samples (FC analysis); 3) internalization kinetics (FC and microscopy analysis); 4) and capacity to induce Antibody-dependent cellular cytotoxicity (ADCC) and/or phagocytosis (ADCP). FJB45 was tested in vivo, using a T-ALL patient-derived xenograft (PDX) mouse model in a phase II-like clinical trial setting.

Results: We identified 4 mAbs with high affinity to hIL-7R: FJB26, FJB29, FJB45 and FJB48. We demonstrate that FJB45 abrogates the effects of physiological levels of IL-7 (≤100 pg/mL) on T-ALL cell viability and proliferation. All mAbs show ADCC potential, and FJB29, FJB45 and FJB48 promote also ADCP. In vivo, FJB45 clearly extends mouse overall survival in 4 out of 10 PDX samples in a phase II-like clinical trial setting.

Conclusions: FJB45 has ADCC- and ADCP-promoting capacity and is effective in vivo. Our studies contribute to the growing evidence that anti-IL-7R mAbs may be a valid therapeutic strategy for IL-7R-positive T-ALL.

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78. Targeting MRP8⁺ Cells as a Tumor-Promoting Inflammatory Vulnerability in Pancreatic Cancer

Authors and Affiliations

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains largely refractory to current immunotherapies, underscoring the urgent need to identify inflammatory circuits that sustain tumor progression and can be therapeutically targeted. Our preliminary data, identify MRP8⁺ cells as a clinically relevant and previously underrecognized inflammatory population in PDAC. Myeloid-related protein 8, MRP8, is a well-established mediator of inflammation in multiple pathologies, yet its functional role in cancer remains less understood. Notably, high intratumoral infiltration of MRP8⁺ cells are strongly associated with poorer overall survival in PDAC patients, suggesting that this population may contribute directly to disease aggressiveness. Using genetically engineered mouse models, we observed a progressive increase in MRP8⁺ cell infiltration across the disease continuum, from pancreatic inflammation to advanced PDAC. This accumulation was evident in acute and chronic inflammatory settings as well as during tumor development, suggesting that MRP8⁺ cells may participate in the pathological transition. Importantly, our findings indicate that these cells are not merely bystanders of inflammation but may act as central regulators of a pathogenic inflammatory cascade that promotes a tumor-supportive microenvironment and accelerates disease progression. This project will investigate the functional and therapeutic significance of MRP8⁺ cells in PDAC. Moving beyond descriptive immunophenotyping, we will define how this inflammatory subset contributes to tumor progression, determine its role across distinct stages of pancreatic disease, and evaluate whether targeting MRP8-dependent inflammatory signaling can impair PDAC development and progression. Stablishing MRP8+ cells as a driver of tumor-promoting inflammation, this project aims to uncover a targetable inflammatory vulnerability in PDAC overcoming inflammatory barriers that limit pancreatic cancer therapy effectiveness.

79. Unravelling Radioresistance in Prostate Cancer Through Epigenetic and Metabolic Perspectives

Authors and Affiliations

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Abstract

Although radiotherapy (RT) is a key treatment for prostate cancer (PCa), overcoming RT resistance remains warranted. Interactions between metabolic reprogramming and epigenetic deregulation – recognized cancer hallmarks – were linked to cancer aggressiveness and therapy response, though their precise role is unclear in PCa.

Cellular glucose/lactate and lipid content were evaluated by colorimetric and red oil assays in two radioresistant (RR) cell lines (22Rv1-RR and C4-2B-RR) generated in-house and the respective parental (P) cell line. Epigenetic and metabolic-related markers were determined at transcript (RT-qPCR) and protein (Western blot) levels.

P cells were treated with lactate, while RR cells were submitted to lactate production and transport inhibition, followed by RR and metabolic phenotype assessment.

RR cells presented increased glucose consumption and lactate production, while lipid content was diminished compared to P cells. RR cells showed higher glycolytic-related markers such as monocarboxylate transporter 4, pyruvate kinase, and lactate dehydrogenase A, as well as the hypoxia-inducible factor 1-alpha, whereas presenting reduced lipidic markers (fatty acid synthase, ATP citrate lyase, and sterol regulatory element-binding protein 1). Importantly, the lactylation mark - H3K18lac - was significantly upregulated in the RR cells, accompanied by decreased histone deacetylases (HDAC1 and HDAC3) and acetyltransferase (KAT2B) expression. Lactate treatment induced a RR phenotype in the P cell line, while lactate inhibition increases RT response.

Our preliminary findings indicate that RR prostate cancer cells undergo metabolic and epigenetic reprogramming through histone lactylation, which may represent a novel therapeutic vulnerability.

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80. A novel pancreatic ductal adenocarcinoma Cell Model of Gemcitabine Resistance

Authors and Affiliations

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Abstract

Gemcitabine-based chemotherapy remains the standard of care for advanced pancreatic ductal adenocarcinoma (PDAC). Although FOLFIRINOX has emerged as an alternative, it provides only modest survival benefits and is associated with higher toxicity. Thus, gemcitabine, alone or in combination, remains widely used. However, its efficacy is often limited by the rapid development of resistance, highlighting the need for new strategies to overcome this challenge.

Our group established a gemcitabine-resistant PDAC cell line (PANC1-CDR) by exposing PANC1 cells to increasing gemcitabine concentrations over six months. Resistance was then confirmed by sulforhodamine B assay. Comparative analyses between sensitive and resistant cells included cell cycle analysis (flow cytometry), colony formation (clonogenic assay), proliferation (BrdU assay), and ultrastructural morphology (electron microscopy transmission) analysis. Proteomic profiling was performed using LC-MS/MS, and differential expression of proteins validated (Western blot). In vivo studies were conducted using PANC1 and PANC1-CDR xenograft mouse models and tumor growth monitored using caliper measurement and 3D ultrasound imaging.

Data showed that PANC1-CDR cells exhibited marked resistance to gemcitabine (GI50 > 150 µM) compared to parental PANC1 cells (GI50 = 0.64 ± 0.09 µM). Resistant cells presented slower growth, reduced G2/M phase distribution, enhanced capacity to form colonies, decreased cell migration capacity, and distinct morphological changes (elongated pseudopodia, preeminent RE cisterna and enlarged vesicles).

PANC1-CDR cells also showed reduced DNA repair and apoptotic proteins, increased expression of proteins involved in ERK and CHI3L1 pathways and decreased expression of proteins from the STAT3 signaling. Consistently, PANC1-CDR xenografts grew more slowly than PANC1 tumors and were resistant to gemcitabine.

Ongoing studies aim to understand the underlying mechanisms of gemcitabine resistance of our developed cell model.

81. T-ALL cells express low BMAL1 but display circadian oscillations, including in PI3K signaling, and can be targeted chronotherapeutically

Authors and Affiliations

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Abstract

Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive childhood malignancy. Despite therapeutic advances, relapsed/refractory disease and treatment-related toxicities remain major challenges. The Circadian Molecular Clock (CMC) regulates homeostasis through 24-hour oscillations of core genes (BMAL1, CLOCK, PER, CRY). Circadian disruption has been linked to cancer, prompting us to explore the role of the CMC in T-ALL and its therapeutic potential.

Materials and Methods: Clock gene expression was analyzed by RNA-seq in primary human thymocytes and T-ALL samples. A conditional Bmal1 knockout mouse model was used to assess leukemia development in vivo following NOTCH1-driven transformation. Circadian oscillations were evaluated in thymocytes, patient samples, and T-ALL cell lines, together with PI3K pathway activity. PI3K inhibitors were administered at distinct circadian timepoints.

Results: BMAL1 is significantly downregulated in T-ALL. While Bmal1 loss does not affect normal lymphoid development, it appears to accelerate leukemia onset in vivo. Despite low BMAL1 levels, T-ALL cells retain circadian oscillations, including rhythmic PI3K signaling. Importantly, sensitivity to PI3K inhibition varies according to treatment timing.

Conclusions: BMAL1 may function as a tumor suppressor in T-ALL. Leukemic cells retain oscillatory capacity and exhibit time-dependent sensitivity to PI3K inhibition, supporting chronotherapy as a strategy to improve treatment efficacy while minimizing toxicity.

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82. IDH1 mutations induce therapeutically exploitable DNA repair and metabolic liabilities

Authors and Affiliations

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Abstract

IDH1 mutations, frequent in gliomas (80%), produce 2-hydroxyglutarate inducing metabolic and epigenetic reprogramming. However, its impact on DNA damage response (DDR) and the resulting therapeutic vulnerabilities remain unclear. This study explored DDR and metabolic vulnerabilities of IDH1-mutant glioma cells and as therapeutic targets.

U87 MG and IDH1-mutant U87 (IDH1m) cells were assessed for DNA damage (micronucleus assay, γH2AX), repair kinetics, and DDR gene expression (qPCR). Metabolic profiling included oxygen consumption rate (OCR), extracellular acidification rate (ECAR) (Seahorse), metabolic dependencies (SCENITH), 13C-Glucose tracing, mitochondrial membrane potential (JC1), and oxidative stress (DCF/DHE). The effects of microglia (HMC3 cells) conditioned medium on metabolism were also evaluated (OCR/ECAR). Therapeutic efficacy of Oligomycin (Oli) and elimusertib (Eli) was tested in monotherapy and with temozolomide (TMZ) by resazurin assay. Synergism (SynergyFinder), antiproliferative, cytotoxic, genotoxic, and metabolic effects were evaluated. 3D models were used for validation.

IDH1m cells showed higher genomic instability, defective repair, and DDR genes downregulation ($p<0.01$). Glycolytic dependence decreased ($p<0.0001$) and mitochondrial metabolism increased, in association with mitochondrial dysfunction and oxidative stress ($p<0.05$). However, microglia CM increased mitochondrial efficiency and glycolytic reserve ($p<0.05$), suggesting a supporting role in metabolic flexibility for IDH1m cells. Oli and Eli were more effective in IDH1m cells, promoting synergistic interactions with TMZ via reduced proliferation, increased p53 expression, genotoxic stress, and restricted metabolic flexibility ($p<0.05$).

IDH1 mutations induce DDR dysfunction and metabolic dependencies that may be modulated by tumor microenvironmental factors. These specific changes make IDH1m cells more vulnerable to DDR and mitochondrial-targeting strategies, sensitizing cells to TMZ.

83. Pyrimidine-based Derivatives as Potent Antitumor Agents Targeting Multidrug Resistance in Cancer

Authors and Affiliations

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Abstract

Pyrimidine-based derivatives are well-established bioactive molecules, with various substitution patterns on the pyrimidine core conferring many properties. Their promising antitumor activity have been attributed to their ability to mimic ATP-binding interactions, which may be useful for the design of new tyrosine kinase inhibitors targeting ATP-dependent proteins. One of those proteins is P-glycoprotein (P-gp), whose overexpression is linked to multidrug resistance (MDR) in cancers, including non-small cell lung cancer (NSCLC) and colorectal cancer (CRC).

The aim of this work was to evaluate the antitumor and anti-P-gp activity of eight novel synthesized pyrimidine-based derivatives, in two counterpart pairs of sensitive and MDR NSCLC and CRC cell lines.

The effect of these pyrimidine-based derivatives on cell growth was evaluated on sensitive A549 NSCLC and DLD1 CRC cells, and on their MDR counterparts, A549-CDR2 and DLD1-TxR, respectively, using the SRB assay. The rhodamine efflux assay was also performed with selected compounds, to evaluate their inhibitory effect on P-gp activity.

Compounds 2, 3, 6 and 8 presented the lowest GI50 concentrations in the sensitive A549 cells (9.8 μM, 8.7 μM, 10.1 μM and 8.2 μM, respectively) and in the MDR counterpart A549-CDR2 cells (12.4 μM, 9.0 μM, 11.6 μM and 7.1 μM, respectively). These compounds also presented the lowest GI50 concentrations in the sensitive DLD1 (10.8 μM, 13.7 μM, 11.5 μM and 8.2 μM, respectively), and in the MDR DLD1-TxR cells (10.6 μM, 14.4 μM, 10.1 μM and 10.2 μM, respectively). In contrast, compounds 1, 4, 5, and 7 demonstrated higher GI50 concentrations (13 - 39 μM) in both pairs of sensitive and MDR cell lines. Moreover, compounds 2 and 3 showed the highest inhibitory effect on P-gp activity, in both MDR CRC and NSCLC cells.

These results suggest that all compounds have similar activity in sensitive and MDR counterpart cell lines. Importantly, compounds 2 and 3 emerge as promising "hit" candidates for further functional and mechanistic studies against MDR in cancer.

84. Repurposing angiotensin-receptor blockers as mechanotherapeutic agents in pancreatic cancer

Authors and Affiliations

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Abstract

Tumor biomechanics plays a critical role in cancer progression, metastasis, and treatment response. Excessive extracellular matrix deposition increases tumor stiffness, limits drug delivery and immune cell infiltration, while intrinsic softness of cancer cells promotes tumor progression and therapy resistance. Angiotensin-receptor blockers (ARBs), widely used to treat arterial hypertension, are being identified as potential modulators of tumor biomechanics. By inhibiting angiotensin II type-1 receptor, often overexpressed in cancer cells and cancer-associated fibroblasts, ARBs have shown potential to improve therapeutic responses.

We are assessing the impact of two ARBs, losartan and valsartan, on pancreatic ductal adenocarcinoma (PDAC) cell biomechanical and biophysical properties and function. Using atomic force microscopy (AFM), we demonstrate that ARBs treatment significantly reduces cell stiffness and cell–cell adhesion forces. AFM imaging also revealed decreased cell surface roughness, without morphological changes. ARBs effect on cell structure is being investigated at the membrane level via fluorescence spectroscopy, with preliminary data indicating altered membrane fluidity. To assess how ARBs-induced biomechanical and structural changes affect phenotype, we are analyzing mechanotransduction pathways and epithelial–mesenchymal transition (EMT) markers by qPCR, observing reduced EMT marker expression with ARBs treatment. Functionally, colony formation assays show a slight decrease in colony area, reflecting ARBs influence on tumor growth.

Extending these observations to clinical application, we will evaluate ARBs biomechanical effects in patient-derived PDAC tissue. Altogether, our work will demonstrate ARBs potential as mechanotherapeutic agents. Given that losartan and valsartan are already clinical approved, their repurpose offers a safe and cost-effective strategy to improve cancer treatment outcomes.

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85. Validating Polygenic Risk Scores for Colorectal Cancer Risk Prediction in the Brazilian population

Authors and Affiliations

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Abstract

Colorectal cancer (CRC) is one of the cancers with the highest incidence and associated mortality worldwide. CRC screening currently relies on a suboptimal one-size-fits-all strategy. While risk-adapted screening, using tools like Polygenic Risk Scores (PRS), shows great clinical potential, its transferability to non-European populations remains a critical limitation. This study aims to evaluate the performance and predictive accuracy of published European-derived CRC PRS in a highly admixed Brazilian cohort.

The cohort comprised 1,458 CRC patients and 2,555 controls. Genotyping was performed using the 850K Axiom Precision Medicine Diversity Array. After quality control, population structure was assessed via Principal Component Analysis (PCA) against a merged reference panel comprising the 1000 Genomes and HGDP, alongside ADMIXTURE ancestry profiling. High-density imputation was conducted with the All of Us reference panel. We retrieved 76 published CRC PRSs from the PGS Catalog and calculated individual profiles using the pgsc_calc. Model performance and associations with CRC risk were assessed using logistic regression, adjusting for age, sex, and PCA covariates.

After QC and imputation, the dataset comprised 11,047,366 variants and 3,679 individuals (1,376 cases and 2,303 controls) with an admixed ancestry profile: European (74.4%), African (17.7%), Native American (5.8%), and Asian (2.1%). Application of PGS004904 demonstrated a significant association with CRC risk, yielding an adjusted OR of 1.51 per SD increase (95% CI 1.41-1.63, p=1.14e-29).

Overall predictive accuracy was modest (adjusted AUC = 0.648). Notably, scores were positively correlated with European ancestry proportions (p=1.14e-11). Systematic evaluation of additional CRC scores is underway.

In conclusion, while European-derived PRS seems to retain significant predictive value in admixed Brazilians, its modest accuracy and correlation with European ancestry may indicate limited clinical transferability.

86. Dissecting spatial niches and immunological cues in glioblastoma for novel biomarkers and precision therapies

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GBM) is characterized by its heterogeneity, which renders standardized treatment ineffective, underlining the need for patient-tailored combination strategies. HOXA9 and WNT6 are oncogenic factors associated with worse GBM prognosis. HOXA9 and WNT6 have been shown to sustain immunoregulatory roles in various contexts, and in GBM we have found an association between these molecules and immune-related markers and processes. Thus, we aim to explore HOXA9/WNT6-driven effects on GBM-immune spatial architecture, unveiling potential therapeutic strategies.

Materials and Methods: GBM patient samples analyzed by Visium (10X Genomics; Ravi et al., 2022; Tirosh et al., 2024) were used to assess co-expression of HOXA9/WNT6 with immune cell types. Additionally, GBM patients from TCGA were stratified into HOXA9/WNT6-high and -low groups for differential expression analysis, with selected genes subjected to literature querying and univariate survival analysis. Pharmacologic inhibition of HOXA9 (direct by DB818, indirect by Buparlisib) and WNT signaling (E7386) was assessed in four paired HOXA9- and WNT6-modulated cell models.

Results: HOXA9/WNT6 show spatial co-expression with multiple immune signatures in GBM, particularly T cells. HOXA9/WNT6 double-high patients present a downregulation of several genes associated with better survival. In vitro, HOXA9-high cells show greater sensitivity to pharmacologic HOXA9 inhibition. WNT signaling inhibition decreases WNT6-driven aggressiveness in overexpression models and acts synergistically with WNT6 silencing in knockdown models.

Conclusions: This data begins to shed light on the association between HOXA9/WNT6 and GBM immune landscape and suggests their pharmacologic targeting as a potential therapeutic avenue. Ongoing spatial transcriptomics and combination treatment studies, using co-cultures with hPBMCs and humanized in vivo models, will further explore this axis, potentially contributing toward more effective GBM care.

87. WNT6 modulates extracellular vesicle biogenesis and cargo loading in glioblastoma: implications for liquid biopsies

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by poor prognosis and limited treatment options. Our team showed that WNT6, a Wnt signaling ligand, is overexpressed in GBM and associated with tumor aggressiveness. Notably, WNT6 was highly enriched in extracellular vesicles (EVs) derived from GBM primary cells. Given the role of EVs in intercellular communication and their potential as minimally invasive liquid biopsy tools, this work explores the biological and clinical relevance of WNT6-driven EV signaling in GBM.

Methods: GBM cell lines with genetically altered WNT6 expression were used. Additionally, plasma from GBM (n=20) and lower grade glioma (n=14) patients were collected from Hospital de Braga. EVs were isolated from conditioned medium (size exclusion chromatography) and plasma (precipitation) and characterized by nanoparticle tracking analysis and western blot. Transcriptomic (RNA-seq) and proteomic (MS/MS) profiles of cells and EVs were assessed by differential expression and pathway analyses, including GO enrichment and GSEA. WNT6 levels in EVs were quantified by western blot, ddPCR and ELISA.

Results: RNA-seq and MS/MS analyses of WNT6-high cells revealed enriched EV and exosome-related signatures. Consistently, WNT6-high cells released more EVs per cell compared to controls. This effect was reflected in patient samples, where plasma EV concentration was increased in WNT6-high tumors, highlighting a link between WNT6 and EV biogenesis. Furthermore, WNT6 reshaped EV transcriptomic and proteomics cargo, with enrichment in oncogenic pathways. Interestingly, WNT6 mRNA and protein were detected in EVs from GBM cells and patient plasma, closely mirroring its cellular and tumoral expression levels, respectively.

Conclusion: WNT6 modulates EV release and shapes oncogenic EV cargo in GBM. Additionally, its detectability in circulating EVs, reflecting tumor expression levels, supports its potential as a liquid biopsy biomarker in glioma.

88. Liquid-biopsy Extracellular Vesicles Signatures of Probable Sarcopenia in Gastric Cancer: a Proteomic Discovery and Flow-Cytometry Validation Study

Authors and Affiliations

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Abstract

Sarcopenia, a condition characterized by accelerated loss of muscle strength and muscle mass or quality, affects approximately one third of Gastric Cancer (GC) patients and is independently associated with post-operative complications, chemotherapy intolerance and reduced survival. Current diagnostic tools are not universally available, and serum biomarkers lack sensitivity for early detection. As such, minimally invasive biomarkers are needed for detection of sarcopenia. Plasma-derived extracellular vesicles (EVs) carry tissue-specific cargo and can be easily isolated from liquid biopsies. To identify a candidate biomarker of clinical relevance, we explored the proteomic signature of plasma-derived EVs in patients with probable sarcopenia (PS) versus non-sarcopenic (NS) patients.

A cohort of 38 patients with gastric/gastroesophageal-junction adenocarcinoma was stratified by EWGSOP2 handgrip dynamometry into PS and NS groups (n=19 PS, n=19 NS). Plasma-EVs from a discovery subset (n=5 PS, n=5 NS) were isolated by size-exclusion chromatography (SEC) combined with ultrafiltration, characterized according to MISEV guidelines by nanoparticle tracking analysis and transmission electron microscopy, and profiled by LC-MS/MS. A selected candidate protein, SERPINB3, was analyzed by nano-flow cytometry in an independent cohort (n=10 PS, n=15 NS), at both diagnosis and after FLOT neoadjuvant chemotherapy.

PS patients displayed impaired performance across functional domains. EV proteomics revealed a discriminative signature of proteins linked to lysosomal/proteolytic and mitochondrial-stress axes. SERPINB3 was significantly elevated in PS at diagnosis ($p<0.05$), was independent of routine inflammatory and nutritional markers, and decreased ~45% after neoadjuvant chemotherapy.

Non-invasive Plasma-EV profiling identified a novel proteomic signature of sarcopenic patients in GC, with potential as a complementary tool for pre-operative risk stratification and treatment response monitoring.

89. Therapeutic targeting of ETV1/ETV4 oncogenic signalling reshapes the tumour microenvironment by modifying vascular architecture in vivo

Authors and Affiliations

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Abstract

Introduction: Prostate cancer (PCa) is the second most frequently diagnosed cancer and a leading cause of cancer-related mortality in men. Genomic rearrangements leading to overexpression of the ETS transcription factors ETV1 or ETV4 occur in 10-15% of cases, defining aggressive molecular subtypes with poor prognosis. Recently, we uncovered EGFR+STAT3 co-inhibition as a potential therapeutic approach for this molecular subtype using in vitro models. However, its impact in tumour microenvironment (TME) remodelling remains unclear. In this study, we leverage the chick embryo chorioallantoic membrane (CAM) as an in vivo platform evaluate how EGFR+STAT3 co-inhibition modulates ETS-dependent TME architecture.

Material and methods: Matrigel-embedded PC3-derived models with knockdown of ETV1, ETV4, or both, were grafted onto CAM at ED8. At ED13, tumours were treated with Erlotinib+TTI-101 or vehicle for 48h. Tumour perimeter was determined in ovo using ImageJ, while ex ovo analyses of the tumors' vasculature was performed using the IKOSA software. Excised tumours were processed for protein analysis using LC-MS/MS and IHC.

Results: Baseline analyses revealed ETS-dependent modulation of microtumours' vascular architecture, characterized by differences in vessels length, thickness, and branching patterns. EGFR+STAT3 co-inhibition increased vessel length and thickness in ETV1 or ETV4-overexpressing tumours, with no effect in those with double-knockdown, suggesting an ETS-dependent impact in vascular architecture remodelling. Preliminary analysis of LC-MS/MS data unveiled chicken Lysozyme C in tumours' protein extracts, supporting therapeutic-driven engagement of innate immune response in ETS-associated TME remodelling.
Conclusions: Oncogenic ETV1 and ETV4 signalling differentially regulate vascular architecture in vivo. Combined inhibition of EGFR and STAT3 reshapes ETS-dependent TME vasculature, potentially increasing innate immune response.

90. Combined targeting of PTEN-regulating microRNAs reduces aggressiveness of clear cell renal cell carcinoma

Authors and Affiliations

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Abstract

Introduction: The therapeutic resistance and variable prognosis in recurrent clear cell renal cell carcinoma (ccRCC) limit therapeutic options of patients. Extracellular vesicles (EVs) have emerged as therapeutic tools due to their role in transporting microRNAs (miRNAs) that regulate gene expression and tumor progression. Previously, we identified an EV-derived miRNA profile (hsa-miR-200c-3p, hsa-miR-25-3p and hsa-miR-301a-3p) that promotes ccRCC progression via PTEN inhibition. Thus, the study evaluates their therapeutic potential in 2D and 3D models.

Material and Methods: EVs from 786-O cells were isolated and characterized by NTA, TEM, and ELISA. Cells were transfected with three miRNA inhibitors using lipofectamine in 2D and 3D cultures. Its effect was assessed by RT-qPCR and western blot for miRNA/PTEN expression, alongside proliferation, migration, metabolic capacity and spheroid morphology analysis. Then, EVs from HEK293T cells were electroporated with miRNA inhibitors and add to 786-O spheroids, followed by functional analysis.

Results: Combined miRNA inhibition using lipofectamine increased PTEN expression, resulting in a decrease in tumor proliferation and migration in 2D, and a reduction in spheroid size and metabolic capacity in 3D. By preliminary study with electroporation, functionalized EVs reduced miRNA expression and increased PTEN mRNA expression in ccRCC spheroids.

Conclusions: These miRNAs are promising therapeutic targets in ccRCC. Their combined inhibition reduces tumorigenic features, supporting personalized treatment strategies.

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91. Uncovering the role of a Ca2+-dependent phospholipid binding protein in glioblastoma recurrence

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Abstract

Glioblastoma (GB) is the most common and aggressive primary malignant brain tumor in adults, characterized by rapid growth, diffuse infiltration into surrounding tissues, and a highly heterogenous molecular and histological profile. Despite standard of care treatment, including surgical resection, radiotherapy and chemotherapy with temozolomide (TMZ), patient prognosis remains poor, with median survival of 15 months. Disease relapse occurs within 1 year and is often driven by TMZ resistance, with only 10% of patients surviving 5 years after diagnosis. This underscores the urgent medical need for novel therapeutic strategies tackling resistance mechanisms.

We previously identified, a Ca2+-dependent phospholipid binding protein (CaPBP), as upregulated in recurrent GB, being correlated with worse patient survival and increased proliferation in vitro. Here we hypothesized that high levels of CaPBP in GB tumors enhance resistance to therapy, contributing to tumor progression and recurrence.

The effect of CaPBP on TMZ response was evaluated in vitro using the U87 GB cell line overexpressing its coding gene (OE-CaPBP). Investigation into relevant signalling pathways by western blot revealed that OE-CaPBP cells have increased levels of phosphorylated S6 compared to control cells, suggesting activation of mTOR pathway. Notably, these cells also present an impaired response to TMZ in vitro.

In vivo studies showed that OE-CaPBP tumors have delayed growth both in orthotopic and subcutaneous GB mouse models, compared to control tumors. Histopathological analysis of subcutaneous tumors demonstrated that OE-CaPBP tumors have altered morphology, with more necrotic areas and decreased angiogenesis, features that resemble the human disease. Importantly, TMZ efficacy is impaired in subcutaneous tumors with high levels of CaPBP, supporting in vitro data.

Overall, these results suggest that CaPBP is a mediator of TMZ resistance in GB.

Note: CaPBP cannot be disclosed at this time due to IP considerations.

92. Association between piperacillin/tazobactam duration and risk of severe neutropenia: a retrospective cohort study protocol

Authors and Affiliations

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Abstract

Introduction: Piperacillin/tazobactam is a widely used broad-spectrum β-lactam antibiotic in hospital settings. Despite its clinical effectiveness, its use has been associated with hematological adverse events, including severe neutropenia, which may increase susceptibility to infections. Evidence suggests that prolonged exposure may increase this risk. However, the relationship between treatment duration and neutropenia remains insufficiently characterized in real-world clinical practice.

Materials and Methods: A retrospective cohort study protocol will be conducted including adult patients (≥18 years) hospitalized with severe urinary tract infection and treated with intravenous piperacillin/tazobactam in two hospitals in Porto, Portugal, between 2023 and 2025. Data will be collected from clinical records, including demographic, clinical, and hematological parameters. Severe neutropenia will be defined as an absolute neutrophil count ≤500 cells/mm³. The primary outcome is the incidence of severe neutropenia according to treatment duration. Secondary analyses will include time to onset of neutropenia, cumulative dose, and hematological recovery after treatment discontinuation. Potential confounders, including age and concomitant medications associated with neutropenia, will be addressed through stratified analyses.

Results: It is expected that prolonged exposure to piperacillin/tazobactam will be associated with a higher incidence of severe neutropenia. The study is anticipated to characterize the temporal relationship between treatment duration and onset of neutropenia, contributing to the identification of patients at increased risk.

Conclusions: This study will address an important gap in post-marketing safety data regarding piperacillin/tazobactam. The findings are expected to support safer prescribing practices and optimize hematological monitoring strategies in hospitalized patients.

93. Biomarkers and pathophysiology of pulmonary fibrosis: perspectives from translational research and biobank

Authors and Affiliations

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Abstract

Introduction: Pulmonary fibrosis is a progressive and heterogeneous condition characterized by excessive deposition of extracellular matrix and deterioration of pulmonary function. Predicting disease progression remains a challenge, and the study of mechanisms and identification of reliable biomarkers are major steps in understanding these complex diseases. Advances in translational research have improved understanding of the development of this pathology and its clinical applications.

Materials and Methods: A narrative review was conducted to synthesize current evidence on mechanisms underlying pulmonary fibrosis and the role of biomarkers in disease progression. Emphasis was given to studies exploring epithelial injury, fibroblast activation, and extracellular matrix remodeling, as well as circulating and molecular biomarkers associated with these processes. The role of biobanking in biomarker discovery and validation was also examined.

Results: Current evidence indicates that multiple biological pathways contribute to pulmonary fibrosis, including epithelial injury, dysregulated repair, and persistent fibroblast activation, influenced by genetic, environmental, and behavioral factors. Several biomarkers, such as markers of epithelial injury and fibrotic activity, have been associated with disease progression and prognosis. Biobanking initiatives allow for the systematic collection of biological samples and clinical data from patients with pulmonary fibrosis, facilitating the identification and validation of new biomarkers and supporting translational research.

Conclusions: Integrating pathophysiological knowledge with biomarker research is essential to advance understanding of pulmonary fibrosis. Biobanks play a key role in this process, enabling high-quality studies. These approaches can contribute to better risk stratification and development of personalized therapeutic strategies.

94. Targeting Pancreatic Cancer with Isoquinolinequinone N-Oxides: Preclinical Evidence of Antitumor Activity

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the 12th most frequently diagnosed cancer and the 6th leading cause of cancer-related death worldwide, with over 500,000 new cases and 470,000 deaths reported in 2022. Its poor prognosis is mainly driven by intrinsic and acquired resistance to therapy, leading to mortality rates exceeding 90%. Current treatments rely largely on gemcitabine, alone or combined with paclitaxel, however, resistance frequently develops during prolonged treatment, highlighting the urgent need for new agents capable of overcoming chemoresistance. Previously, isoquinolinequinone (IQQ) N-oxides demonstrated significant antitumor activity in both sensitive and multidrug-resistant lung and colorectal cancer models. In this work, we evaluated their efficacy in PDAC sensitive and resistant cell lines. MiaPaCa-2, Capan-1, BxPC-3, Panc-1, and gemcitabine-resistant Panc-1-CDR cells were treated with IQQ N-oxides (RK1-RK9) for 48 h, and cell growth was assessed using the SRB assay. Long term clonogenic potential was evaluated by colony formation assay after 2 and 6 days, and anti-migratory effects were assessed by wound healing assay over 48 h. RK2 and RK3 emerged as the most active derivatives, with GI50 values ranging from 0.80 to 2.35 μM across all PDAC cell lines, and maintained activity in Panc-1-CDR cells (GI50 1.41 and 1.14 μM). Both compounds markedly reduced clonogenic survival in a concentration and time dependent manner, achieving near complete inhibition by day 6 in Panc-1 cells. At 1 μM, RK2 and RK3 significantly impaired migration, limiting wound closure to 60% and 51%, respectively, compared to 80% in controls. Overall, RK2 and RK3 effectively inhibit PDAC cell growth, including in gemcitabine-resistant models, while suppressing key malignant features such as self-renewal and migration, supporting their potential as promising therapeutic candidates.

95. Development of a spectral flow cytometry panel for immunological biomarker assessment in CAR-T cell therapy

Authors and Affiliations

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Abstract

Immunotherapy is at the forefront of cancer treatment, with chimeric antigen receptor T (CAR-T) cell therapy emerging as a promising approach in hematologic malignancies. Despite encouraging clinical outcomes, the identification of robust biomarkers to predict response and monitor toxicity remains a major challenge. Spectral flow cytometry (SFC) enables high-dimensional immune profiling and is a powerful tool for biomarker studies.

Thus, we aim to develop and optimize SFC-based panels for high-dimensional immunophenotyping of patients undergoing CAR-T therapy. This work is part of the CARTMatters Project (COMPETE2030-FEDER-01477200), a national consortium focused on capacity building for CAR-T cell production in Portugal, integrating tasks on clinical patient profiling and starting material characterization.

Here, our main goal is to conduct comprehensive immunophenotyping using SFC on peripheral blood from patients: (1) with B-cell lymphoma eligible to initiate CAR-T therapy, with samples collected before and after CAR-T cell infusion; (2) who underwent leukapheresis for CAR-T cell manufacturing.

Three different SFC panels were designed and are being optimized using the Cytek Aurora cytometer. Each panel allows in-depth immunophenotyping of T, B and myeloid cell populations, different cell subsets, and several markers.

The T-cell panel includes a specific marker for CAR-T detection, and enables characterization of activation, exhaustion, senescence and immune checkpoints markers. The myeloid panel assesses myeloid-derived suppressor cells, monocytes, and dendritic cells, while B-cell panel enables the identification of different subsets and disease-associated populations.

Multiparametric SFC data will be integrated with clinical information, enabling a broader characterization of patients' immune status, contributing to the identification of treatment response biomarkers and improve the understanding of immune mechanisms upon CAR-T therapy.

96. In-depth serum glycoproteomics reveals stage-dependent a2,6-sialylation and systemic prothrombotic signalling in gastric cancer

Authors and Affiliations

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Abstract

Gastric cancer (GC) remains a major global health burden, and its incidence is projected to rise in the coming decades. Many patients are diagnosed at advanced stages, limiting therapeutic options and compromising clinical outcomes. The identification of minimally invasive biomarkers for early detection, prognosis and disease monitoring remains an unmet clinical need. Tumours show glycocalyx alterations, including increased α2,6-sialylation driven by ST6GAL1 overexpression, a feature associated with progression, dissemination and poor prognosis. Glycoproteins carrying these altered glycans are released into the bloodstream, making the serum glycoproteome a valuable source of biomarkers.

We performed glycoproteomic profiling of serum samples from GC patients, by SNA lectin enrichment and ion mobility nanoLC-ESI-MS/MS using complementary HCD and HCD-triggered EThcD fragmentation for confident glycosite annotation.

Serum from GC patients showed increased α2,6-sialylation, consistent with ST6GAL1-driven remodelling. This revealed stage-dependent glycoproteomic signatures with potential value for patient stratification. Most altered glycoproteins were not of direct tumour origin. but reflected a non-tumour-derived signature enriched in liver- and immune-associated compartments. These observations support systemic tumour-host crosstalk rather than a purely tumour-secreted signature. GC patients showed glycoproteomic features consistent with a prothrombotic state, including altered complement-related and platelet-associated glycoproteins such as C3, C4A and multimerin-1. Given that terminal sialylation enhances circulatory persistence by limiting hepatic clearance, these alterations may favour the accumulation in the bloodstream.

These findings support risk stratification, closer monitoring of thromboembolic complications, and identification of patients who may benefit from more personalized supportive care.

97. Self-antigen presentation drives T-cell leukemia development in a TCR transgenic mouse model

Authors and Affiliations

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Abstract

T cell acute lymphoblastic leukemia (T-ALL) is a malignancy characterized by developmental arrest of immature T cells, with rapid dissemination of malignant cells. The T cell receptor (TCR), plays an essential role in T cell development, defining T cell fate during positive and negative selection in the thymus. Since many T-ALL cases exhibit TCR on their leukemic cells, we aimed to understand the TCR role in leukemia development, and the impact of leukemic cell antigenic stimulation.

A cohort of Rag2-/- mice expressing transgenic TCR OT-I, were followed for 12-18 months. Around 60% of TCR OT-I mice developed T-ALL as defined by flow cytometry immunophenotyping, characterized by thymic lymphoma, lymphocytosis and dissemination to lymphoid and non-lymphoid organs. We also found that mice with two OT-I transgene alleles had significantly higher T-ALL incidence than mice with one OT-I allele. Of note, mice with two OT-Î alleles had increased proportions of immature thymocytes (DN stage) and TCR+ cells than mice with one allele. Disruption of TCR–MHC class I interaction (B2m KO) impaired T-ALL development, suggesting a proleukemogenic role for basal TCR signaling.

Leukemic and non-leukemic OT-I T cells were stimulated in vitro with OVA to assess their response to activation of the TCR pathway. Unexpectedly, most OT-I leukemic cells poorly upregulated the CD69 activation marker compared to healthy OT-I lymphocytes. To investigate a possible loss of function of the transgenic TCR, these cells were stimulated with anti-CD3 monoclonal antibody and PMA/Ionomycin. Once again, leukemic OT-I cells were less responsive to these stimuli compared to healthy OT-I thymocytes.

Overall, our findings demonstrate that expression of a transgenic TCR can drive T-ALL, with disease onset closely linked to basal TCR signaling and transgene dosage. Leukemic cells exhibited impaired response to TCR stimulation. These results suggest that while basal TCR signaling promotes leukemogenesis, TCR activation becomes dysfunctional in established disease.

98. Beyond mutations: PIK3CA mutant allelic expression imbalance associates with clinical outcome in colon and prostate cancer

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Abstract

Precision oncology has advanced cancer management with stratification of patients beyond histological diagnosis. PIK3CA is among the most frequently mutated oncogenes in cancer, yet its clinical impact is still poorly understood. We previously demonstrated that PIK3CA mutant allelic expression imbalance (mutAEI) is prevalent in breast cancer and associated with patient's survival. Here, we hypothesize that PIK3CA mutAEI occurs in colorectal and prostate cancers and similarly hold prognostic significance

We analysed annotated mutations, RNA-seq and clinical data of PIK3CA-mutated colorectal (TCGA-COAD) and prostate (TCGA-PRAD) tumours, available at the GDC data portal and PCAWG projects. Allelic expression was quantified using a bioinformatic pipeline that reduces wild-type allele mapping bias via STAR+WASP and ASEReadCounter. After excluding hypermutated tumours, samples harbouring multiple PIK3CA mutations and those in the lowest quartile of total read count, 41 colon and 12 prostate tumours were retained for mutAEI and clinical outcome analysis.

PIK3CA MutAEI was detected in 22% of colorectal and 75% of prostate tumours. Higher expression of the wild-type allele was associated with a worse prognosis in colorectal cancer, whereas it was associated with better prognosis in prostate cancer. In colorectal cancer, cis-regulatory effects and DNA allelic content accounted for 47% and 20% of mutAEI, respectively.

Here, we show that PIK3CA MutAEI is a frequent and prognostically relevant phenomenon in colorectal and prostate cancers, identifying subgroups of patients with distinct survival outcomes. These findings suggest that quantifying mutant allele expression provides prognostic information beyond that offered by standard DNA mutation calling. Validation in independent cohorts is warranted to establish mutAEI as a robust biomarker.

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99. Unveiling CD137: A Novel Resistance Axis in Myeloproliferative Neoplasms

Authors and Affiliations

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Abstract

Introduction: BCR-ABL1–negative myeloproliferative neoplasms (MPN) rely on constitutive JAK-STAT signaling, mostly driven by the JAK2V617F mutation. Despite providing significant symptomatic relief, JAK inhibitors (ruxolitinib) fail to achieve disease eradication, with allogeneic transplantation remaining the only curative option. Therapeutic resistance is a major limitation, and we have previously demonstrated that the bone marrow (BM) actively promotes ruxolitinib resistance in MPN cells. Deciphering the molecular determinants underlying BM-mediated protection is therefore critical to identify new therapeutic vulnerabilities.

Materials and Methods: MPN cells were cultured in the presence or absence of BM-derived support and treated with ruxolitinib. Gene expression and signaling pathway activation were assessed by quantitative PCR and Western blotting. CD137 (TNFRSF9) expression was interrogated by flow cytometry and genetically ablated using CRISPR/Cas9. Apoptosis was quantified by Annexin V/7-AAD staining and FSC vs SSC gating.

Results: We identify CD137 (TNFRSF9), a member of the TNF receptor superfamily, as a mediator of BM-driven resistance to JAK inhibition in MPN. BM contact upon ruxolitinib exposure induces both transcriptional upregulation and surface expression of CD137. Functionally, CD137 loss hinders therapeutic response, as CRISPR/Cas9-mediated ablation renders MPN cells completely refractory to JAK inhibition, independently of BM support. Mechanistically, this resistance phenotype is associated with compensatory activation of the PI3K–Akt and MEK-Erk signaling pathways.

Conclusions: Collectively, we identify CD137 as a pivotal regulator of BM microenvironment-driven resistance in MPN, functionally rewiring survival signaling to bypass JAK–STAT inhibition. These results identify CD137 as a novel therapeutic target in MPN disease and provide a strong rationale for combination strategies designed to overcome resistance to ruxolitinib.

100. Microbiome-derived kynurenine: a potential driver of gastric cancer progression

Authors and Affiliations

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Abstract

Introduction: Gastric cancer remains one of the most incident and deadliest cancers worldwide. Helicobacter pylori is the main etiological agent, but increasing evidence, including our own, indicates that the gastric microbiota also contributes to carcinogenesis. The mechanisms remain unclear, though microbiome-derived metabolites are emerging as key mediators. We hypothesize that, during gastric carcinogenesis, the microbiome adopts a distinct metabolic program that generates oncogenic metabolites. We previously developed a machine learning model integrating metatranscriptomics and metabolomics data, identifying several metabolites, including Kynurenine (Kyn). This study aims to investigate the mechanisms by which Kyn contributes to gastric cancer.

Materials and Methods: Untargeted metabolomics was performed on 60 tissues (30 tumours and 30 paired normal tissues). Functional assays in gastric cancer cell lines assessed Kyn effects on cell viability, aryl hydrocarbon receptor (AhR) activity, and downstream gene expression.

Results: A total of 873 metabolites were detected, with 41 of these significantly enriched in GC. Consistent with model predictions, Kyn was significantly elevated in tumour tissues. Analysis of the Cancer Genome Atlas (TCGA) gastric cancer RNA-seq dataset identified 15 bacterial enzymes, encoded by 720 genes, involved in Kyn metabolism. Time-course experiments showed that Kyn enhanced cancer cell viability. In addition, Kyn also promoted gastric epithelial cell invasion in an AhR-dependent manner. We successfully demonstrated that Kyn activated AhR signalling, leading to an increased expression of downstream targets, namely CYP1A1 and MMP1.

Conclusion: Gastric tumours are enriched in Kyn, a microbiome-derived metabolite that enhances cell viability and invasion via AhR activation. These findings highlight Kyn as a potential link between the gastric microbiome and tumour progression.

101. CCL18-expressing macrophages may drive an immunosuppressive and pro-invasive colorectal tumor microenvironment

Authors and Affiliations

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Abstract

Introduction: The immunosuppressive colon cancer tumor microenvironment (TME) is shaped by cancer, immune, stromal cells, and the extracellular matrix. We previously showed that decellularized tumor matrices from colon cancer patients induce macrophage CCL18 secretion. We also showed that this pro-fibrotic and immunosuppressive chemokine promotes cancer cell invasion and is expressed by macrophages at the invasive front of advanced tumors. We now aim to transcriptionally and spatially characterize CCL18-expressing macrophages and dissect their TME interactions.

Materials and methods: We performed advanced mass spectrometry on decellularized tumor and normal matrices. Multiplex Tissue Microarray (TMA) was performed on sections derived from 12 colorectal cancer patients and analyzed using the AIVIA software. Additionally, publicly available single-cell RNA-sequencing (scRNAseq) data, from colorectal cancer patients, together with single-cell spatial transcriptomics (ST) data (AC-ICAM cohort) were analyzed.

Results: By intersecting the proteins with increased expression in tumor dECM with the genes positively correlated with CCL18 we uncovered a 19-protein panel. scRNASeq data evidenced that these genes are mainly expressed by fibroblasts, as the case of FAP, a well-known marker of cancer-associated fibroblasts. TMA's analysis reveals a close proximity between FAP, CD68 and CCL18 at the tumor invasive front. Additionally, CCL18 is expressed by macrophage populations characterized by genes such as SPP1, MARCO and APOE, described as markers of immunosuppressive phenotype. Initial data from the ST analysis reveals that these are spatially close together with MMP12.

Conclusion: In colorectal tumors, FAP displays close spatial association with CCL18+ macrophages. The proximity between SPP1, MARCO, APOE and MMP12 with CCL18+ macrophages may reveal a crosstalk that contributes to the formation of a pro-invasive and immunosuppressive environment, which is currently under investigation.

102. phenotypic heterogeneity in cancer stem cells of non-muscle invasive bladder cancer

Authors and Affiliations

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Abstract

Introduction: Non-muscle invasive bladder cancer (NMIBC) is characterized by high recurrence and progression rates despite standard intravesical Bacillus Calmette-Guérin (BCG) immunotherapy. Cancer stem cells (CSCs) have been associated with therapeutic resistance and tumour recurrence. This study aimed to evaluate the relevance of CSC-enriched populations in NMIBC therapeutic response.

Materials and Methods: RT4 and TCCSUP bladder cancer cell lines were cultured under adherent and sphere-forming conditions. Therapeutic response to cisplatin and gemcitabine was evaluated by MTT assay up to 72h. Cell migration and invasion were evaluated by scratch and transwell assays, respectively. In CSC-enriched populations, sphere-forming capacity and sphere projection area were analysed as indicators of self-renewal, as well as expression of stemness-markers like, CD44, CD24 and CD133 by flow cytometry.

Results: The two cell lines exhibited distinct sensitivities to cisplatin and gemcitabine. TCCSUP cells were less sensitive to cisplatin but more sensitive to gemcitabine compared to RT4 cells. In addition, TCCSUP cells demonstrated faster wound closure and greater invasive capacity than RT4 cells. Sphere-forming cultures showed increased expression of CSC-associated markers relative to adherent cultures. Notably, RT4 cells displayed higher sphere-forming capacity, whereas TCCSUP cells generated fewer spheres that were larger and more irregular in shape.

Conclusions: In conclusion, our results demonstrate that populations enriched in CSCs exhibit characteristics associated with greater aggressiveness, self-renewal capacity, migration and invasion, as well as distinct profiles of sensitivity to the chemotherapeutic agents cisplatin and gemcitabine between cell lines. These differences reinforce the significant role of CSCs in treatment resistance and the biology of NMIBC, supporting their potential as biomarkers or therapeutic targets to overcome the high rate of recurrence and tumour progression.

103. Bioengineered 3D model of invasive breast cancer for studying metabolic reprogramming and drug response

Authors and Affiliations

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Abstract

Breast cancer (BC) is the most frequently diagnosed cancer worldwide and a leading cause of cancer-related mortality in women. Limited therapeutic efficacy and metastatic spread remain major clinical challenges. The tumor microenvironment (TME) strongly influences progression, drug response, and organ-specific metastasis; however, conventional in vitro models often fail to capture these complex dynamics.

We developed a scaffold-based 3D model composed of a tumor core embedded in a collagen scaffold. Unlike pre-mixed models where cells are pre-dispersed in a matrix, our system maintains tumor cells as a defined core that actively invades the surrounding scaffold. The platform accommodates different BC cell lines (MDA-MB-231, MDA-MB-231 BrM2, and SKBR3) in single-culture or co-culture with fibroblasts and monocytes, enabling key tumor–stroma–immune interactions. This system has been validated for microscopy, flow cytometry, and metabolomics profiling.

Preliminary data from targeted metabolomics of BC cells in single-culture revealed a distinct redox and glucose-handling phenotype compared with conventional 2D models. This is consistent with a reprogramming of cellular metabolism under the oxygen and nutrient gradients inherent to the scaffold-based 3D architecture.

In co-culture, we observed monocyte differentiation into macrophages with tumor-specific polarization, suggesting the model effectively recapitulates TME-driven immune modulation. Functionally, the MDA-MB-231 cells in the 3D co-culture system when treated with doxorubicin (1.5 µM) exhibited a 30% increase in overall viability compared with single-culture controls. This resistance to standard-of-care chemotherapy supports the role of microenvironment-mediated therapeutic resistance and demonstrates the relevance of this platform for drug screening applications.

These results highlight the potential of this platform to bridge the gap between conventional in vitro assays and clinically relevant tumor responses.

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104. Development of novel 99mTc-Radiopharmaceuticals for Prostate Cancer

Authors and Affiliations

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Abstract

Introduction: Over the last years, there has been a significant interest in the design of new theranostic radiopharmaceuticals targeted at the Prostate-Specific Membrane Antigen (PSMA), which is overexpressed in the majority of prostate cancer (PCa) and its metastases. Therefore, PSMA-targeted radiopharmaceuticals such as 68Ga-PSMA-11 (locametzTM) and 177Lu-PSMA-617 (PluvictoTM) have opened new avenues for PCa diagnosis and treatment, respectively. However, despite the implementation and importance of PET modality, 99mTc-radiopharmaceuticals continue to play a crucial role in nuclear medicine worldwide. Therefore, the research towards the development of new 99mTc-radiopharmaceuticals still needs to be reinforced. Having this in mind, we have designed a novel [99mTc]Tc-PSMA complex based on pyrazolyl-diamine chelators carrying a PSMA inhibitor for potential application in diagnostic imaging by SPECT. Herein, we report on the synthesis, characterization and preliminary biological evaluation of the designed [99mTc]Tc-PSMA radioligand.

Materials and Methods: The synthesized ligand was radiolabeled with 99mTc. The biological evaluation of the [99mTc]Tc-PSMA complex included cellular uptake and internalization and PSMA-blocking studies in PSMA-positive (PC3 PIP) and PSMA-negative (PC3-FLU) cells.

Results: The designed pyrazole-diamine ligand bearing a PSMA inhibitor was synthesized and efficiently radiolabeled with 99mTc affording a [99mTc]Tc-PSMA complex with high radiochemical purity (RCP) and high specific activity. Furthermore, the PSMA-targeted 99mTc-radiocomplex display high and specific cellular uptake and internalization in the PSMA-positive PC3 PIP cells while showing negligible uptake in the PSMA-negative cells. This strong selectivity was further corroborated by receptor-blocking studies.

Conclusions: We successfully developed a 99mTc-PSMA complex that showed high and specific internalization in PSMA+ PCa cells. Further biological evaluation is currently underway.

105. Unveiling Mechanisms of Intercellular Communication Mediating Drug Resistance in Non-Small Cell Lung Cancer

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Abstract

Non-small cell lung cancer (NSCLC) is frequently driven by EGFR mutations. Although EGFR-targeted therapies (EGFR-TKIs) improve clinical outcomes, resistance inevitably occurs. Despite being the triggering event, our group observed that resistance-associated mutations are present only in a subset of relapsing tumor cells, suggesting that resistance cannot be fully explained by clonal selection alone and supporting a more dynamic model involving intercellular communication.

Human Erlotinib-resistant H1975 and Erlotinib-sensitive HCC827 cell lines, were used both in vitro and in a co-inoculation immunodeficient mouse model, to study intercellular communication dynamics, the transcriptomic, proteomic and epigenetic profile of tumors comprised of therapy sensitive cells before and after development of resistance was assessed. To elucidate the nature of the intercellular communication that leads to the acquisition of mutation-independent resistance to EGFR-TKIs EV and EV-depleted fractions of conditioned media from these cells will be characterized.

Exposure to conditioned medium from resistant cells and co-inoculation in vivo leads to a faster and more stable resistance phenotype. This process was associated with extensive transcriptomic reprogramming, including enrichment of endocytosis pathways marked by Caveolin-1 overexpression, further validated in human samples. PI3K/AKT signaling emerged as a central mediator with significant transcriptomic and proteomic alterations, whose pharmacological inhibition restores EGFR-TKI sensitivity. Epigenetic profiling revealed that the resistance phenotype is associated with a shift towards a more hypomethylated profile characterized by the overexpression of PI3K/AKT and MAPK related genes.

These findings support a model in which resistance to targeted therapy arises through paracrine-driven reprogramming of therapy-sensitive cells, highlighting intercellular communication as a key mechanism and potential therapeutic target in NSCLC.

106. Unveiling the molecular cargo of cancer-derived extracellular vesicles

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Abstract

Introduction: Extracellular vesicles (EVs) are nanosized particles secreted by cells that mediate intercellular communication in cancer. They carry molecular cargo reflecting the physiological and pathological state of their cell of origin, including nucleic acids, proteins, lipids, and glycans. Cancer-derived EVs often display aberrant glycosylation patterns associated with malignant progression, making them a promising source of cancer-related biomarkers. Their stability in biofluids and accessibility through minimally invasive sampling further support their clinical potential. In this work, we investigated the molecular composition of cancer-derived EVs, focusing on their proteomic and glycomic signatures.

Materials and methods: EVs were isolated from multiple biological sources, including plasma from healthy individuals, cancer patients, and cancer cell models. Glycan detection was performed using a panel of lectins and glycan-binding antibodies, while mass spectrometry-based approaches enabled in-depth proteomic and glycomic characterization.

Results: Our analyses revealed distinct, source-dependent molecular signatures at both the protein and glycan levels. EVs from plasma and cancer cell models exhibited cancer-associated alterations that reflected tumor origin and disease context, while also identifying candidate markers linked to pathological states.

Conclusions: Overall, the EV multi-omic profiling represents a promising strategy to further advance precision oncology and deepen our understanding of cancer pathophysiology.

107. Unlocking the therapeutic potential of extracellular vesicles for immunotherapy in triple-negative breast cancer

Authors and Affiliations

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Abstract

Dendritic cells (DCs) are antigen-presenting cells involved in tumor antigen presentation to naive T cells. DC-derived extracellular vesicles (DC-EVs) promote tumor antigen-specific responses, present MCH complexes at the surface and resist to immunosuppression. Nevertheless, DC-EVs immunotherapeutic potential is not fully explored. We propose to unveil the DC-EVs role as an immunotherapy for triple-negative breast cancer, orphan of effective therapies.

Two strategies were developed to do MDA-MB-231 TNBC lysates: freeze/thaw plus sonication (FT+S) and sonication (S). Monocytes from healthy blood donors were cultured with IL-4 and GM-CSF. FT+S and S lysates with MPLA were added at day 4 to induce DCs activation. DCs-EVs were isolated by size exclusion chromatography (SEC) and characterized by Western Blot, NTA and TEM. Pre-educated DCs, conditioned media (CM) from pre-educated DCs and DC-EVs potential to activate T cells was evaluated by flow cytometry for specific T cells markers.

Tumor cell lysates FT+S and S expressed immunological cell death markers (HMGB1, HSP90, Calregulin). DCs phenotype was confirmed by flow cytometry (CD11c, CD86, CD40, HLA-DR), suggesting DCs activation with cell lysates plus MPLA, with increased expression of CD86 and HLA-DR. ELISA of CM revealed an increase of IL-6 and IL-12p40 expression. We observe a peak corresponding to EVs size (~130.5 ± 13.0 nm) by NTA, cup shaped particles by TEM, and expression of EVs and DC-EVs markers (CD9, CD81, CD63, Alix, CD86, HLA-DR), suggesting successful EVs isolation. Pre-educated DCs are able to induce T cells activation, with increase in CD8+CD69+ T cells expression and of IFN-γ production in CM. Moreover, CM from pre-educated DCs is able to activate T cells, with increase in CD8+CD69+ T cells expression. Studies to evaluate DC-EVs potential to activate T cells are being conducted, together with proteomics and toxicity assays to evaluate TNBC cell death upon exposure to pre-educated DCs CM and DC-EVs.

Our data shows that tumor cell lysates with MPLA activated DCs and were able to induce CD8+ T cell activation. However, DC-EVs immunomodulatory potential is still not clear. Further studies will dissect the immunomodulatory potential of DC-EVs, shedding light on new possibilities for immunotherapy for TNBC.

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