6TH ASPIC INTERNATIONAL CONGRESS

UNIVERSIDADE DO ALGARVE • FARO

29-30 APRIL, 2024

PROCEEDINGS BOOK

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LETTER OF WELCOME

Dear Colleagues and Friends,

As Presidents of the **6th ASPIC International Congress**, and on behalf of the Organizing and Scientific Committees, it is with great enthusiasm that we invite you to attend this congress, which will be held on **April 29th and 30th, at the University of Algarve, Campus de Gambelas, Faro, Portugal**.

This is the first time that ASPIC is organizing its international congress outside Lisbon and Porto and we are very happy and confident about our choice. Faro is a welcoming city, with a warm climate and people, and with excellent cancer research groups. ASPIC is very grateful to the University of Algarve for hosting us in 2024 and we promise another rewarding meeting and an exceptional opportunity to share basic, translational, and clinical data, as well as to develop networking with professionals involved in cancer research.

The Congress Scientific Programme will feature lectures performed by invited speakers from ASPIC, ASEICA, and EACR, as well as four symposiums that cover a broad range of topics led by selected experts sharing their knowledge and most recent advances, including selected oral presentations from the abstracts submitted. We also expect a big and participative Poster Session.

You are invited to take an active part in this conference, which we believe will be an outstanding scientific event. We hope that you will take the opportunity to benefit from the exciting scientific programme and from new contacts and collaborations. During the meeting, there will be great opportunities for young scientists to meet the experts in the field, and we aim to create an open and engaging environment for discussion.

We look forward to welcoming you at the fantastic city of Faro.

Joana Paredes, Ana-Teresa Maia and **Fernando Schmitt**

(Presidents of the 6th ASPIC International Congress)

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Bruno Costa

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Jorge Lima

Mónica Teotónio Fernandes

Sara Ricardo

CONGRESS PROGRAMME

29TH APRIL, 2024

09:00 · OFFICIAL OPENING

Joana Paredes (ASPIC, i3S, Porto, PT)
Ana-Teresa Maia (RISE@CINTESIS – UAlg, Faro, PT)

09:30 · ASPIC LECTURE – PROGNOSIS OF BREAST CANCER ASSOCIATED WITH THE PORTUGUESE BRCA2 FOUNDER MUTATION (BRCA2P)

Fátima Vaz (IPO-Lisboa, Portugal)

10:00 · ASPIC LECTURE – POPCARE: A NATION-WIDE PLATFORM TO SPEED UP PRECISION MEDICINE IN PEDIATRIC ONCOLOGY

Jorge Lima (Ipatimup/i3S, Porto, PT)

10:15 · USA-PT LECTURE – UNDERSTANDING & TAMING PANCREAS CANCER

Ronald DePinho (MDAnderson, Houston, USA)

11:00 · COFFEE BREAK

SYMPOSIUM I – TUMOUR GENOMICS & EVOLUTION

Chair: **Joana Xavier** (RISE@CINTESIS -UAlg, Faro, PT) & **Jorge Lima** (Ipatimup, Porto, PT)

11:30 · FLUCTUATING METHYLATION FOR LINEAGE TRACING IN CANCER

Trevor A Graham (ICR, London, UK)

12:00 · SELECTED ORAL COMMUNICATION

EXPLOITING THE RELEVANCE OF WNT6 IN EXTRACELLULAR VESICLES OF GLIOBLASTOMA: CLINICAL AND MOLECULAR INSIGHTS

Helena M. Figueiredo (ICVS, Braga, PT)

12:15 LEUKEMIA STEM CELLS: SEEK AND DESTROY

Alice Giustacchini (Human Technopole, Milan, Italy)

12:45 · SELECTED ORAL COMMUNICATION

SPHINGOSINE KINASE ACTIVITY IS ESSENTIAL FOR IL-7R-MEDIATED SIGNALING IN ACUTE LYMPHOBLASTIC LEUKEMIA, BUT NOT IN HEALTHY LYMPHOCYTES

Marta B. Fernandes (IMM, Lisbon, PT)

13:00 · LUNCH

14:00 · POSTER SESSION

16:00 · COFFEE BREAK

SYMPOSIUM II – TUMOR METABOLISM & EPIGENETIC CONTROL

Chairs: **Bibiana Ferreira** (ABC-Ri, Faro, PT) & **Carmen Jerónimo** (IPOP, Porto, PT)

16:30 · NUTRIENT SIGNALING TO MTOR AT THE CROSSROADS OF CANCER AND AGING

Alejo Efeyan (CNIO, Madrid, Spain)

17:00 · SELECTED ORAL COMMUNICATION

LACTATE IN THE EPIGENETIC LANDSCAPE RECODING: TOWARDS NEW THERAPEUTIC STRATEGIES IN RENAL CELL CARCINOMA

Vera Miranda-Gonçalves (CI-IPOP, Porto, PT)

17:15 · MUTAGENESIS, LESION SEGREGATION, AND CANCER GENOME FORMATION

Duncan Odom (DKFZ, Heidelberg, Germany)

17:45 · SELECTED ORAL COMMUNICATION

NANO-IMMUNOTHERAPY COMBINATION TO REGULATE IMMUNE PROFILING AND TUMOR-ASSOCIATED STROMA IN PANCREATIC CANCER

Liane Moura (iMed.ULisboa, Lisbon, PT)

18:00 · ASPIC GENERAL ASSEMBLY AND ELECTIONS

20:00 · CONGRESS DINNER

30TH APRIL, 2024

PLENARY LECTURES

Chairs: **Wolfgang Link** (FMCB, Faro, PT) & **Sérgio Dias** (IMM, Lisbon, PT)

09:00 · ASEICA LECTURE – UNDERSTANDING AND PREDICTING PROSTATE CANCER AGGRESSIVENESS THROUGH THE DECONSTRUCTION OF CELL SIGNALING AND METABOLISM

Arkaitz Carracedo (CICbioGUNE, Bizkaya, Spain)

09:45 · EACR LECTURE – THE METABOLIC CROSS-TALK BETWEEN T CELLS AND TUMOR CELLS IN MELANOMA-DERIVED METASTASIS

Ilaria Elia (KU, Leuven, Belgium)

10:30 · COFFEE BREAK

SYMPOSIUM III – IMMUNOTHERAPIES & MICROENVIRONMENT

Chairs: **Daniel Bandarra** (CHUA, Faro, PT) & **João Nuno Moreira** (FFUC, Coimbra, PT)

11:00 · EXPANDING THE CANCER IMMUNOTHERAPY TOOLBOX

Noel de Miranda (LUMC, Leiden, Netherlands)

11:30 · SELECTED ORAL COMMUNICATION

IMMUNOSURVEILLANCE SHAPES THE TUMOR GENETIC LANDSCAPE SELECTING AGAINST PUTATIVE IMMUNOGENIC MUTATIONS

Helena Xavier-Ferreira (i3S, Porto, PT)

11:45 · INTEGRATED MULTI-OMIC ANALYSIS OF ADAPTIVE IMMUNOSURVEILLANCE IN EARLY AND METASTATIC BREAST CANCER

Stephen Sammut (ICR, London, UK)

12:15 · SELECTED ORAL COMMUNICATION

EXOSOMES DEFINE A LOCAL AND SYSTEMIC COMMUNICATION NETWORK IN HEALTHY PANCREAS AND PANCREATIC DUCTAL ADENOCARCINOMA

Sónia A. Melo (i3S, Porto, PT)

12:30 · LUNCH

13:30 · POSTER SESSION

SYMPOSIUM IV – TRANSLATIONAL ONCOLOGY

Chairs: **Ana Teresa Maia** (RISE@CINTESIS – UAlg, Faro, PT) & **Cláudia Faria** (IMM, Lisbon, PT)

15:30 · THE IMMUNE LANDSCAPE OF BREAST CANCER DURING PREGNANCY

Elena Guerini Rocco (IEO, University of Milan, Milan, Italy)

16:00 · SELECTED ORAL COMMUNICATION

THE ZAVATAR-TEST FORECASTS PATIENT'S TREATMENT OUTCOME IN COLORECTAL CANCER: A CLINICAL STUDY TOWARDS PERSONALIZED MEDICINE

Bruna Costa (Champalimaud Foundation, Lisbon, PT)

16:15 · RANK PATHWAY DRIVEN THERAPEUTIC OPPORTUNITIES IN BREAST CANCER

Sandra Casimiro (IMM, Lisbon, PT)

16:45 · SELECTED ORAL COMMUNICATION

A 3D TUMOR-ON-CHIP PLATFORM TO STUDY INTRAVASATION

Carolina Morais (IMM, Lisbon, PT)

17:00 · COFFEE BREAK

17:30 · AWARDS & CLOSING SESSION

Fernando Schmitt (RISE@CINTESIS – UP, Porto, PT)

ASPIC Lecture – Prognosis of Breast Cancer Associated with the Portuguese BRCA2 Founder Mutation (BRCA2-P)

Author and Affiliation

Fátima Vaz

On behalf of BRCA2-P investigators

Abstract

BRCA1 and BRCA2 associated BC accounts for 25–30% of familial BC and 3% of all BC. Most populations exhibit a wide spectrum of variants in both BRCA genes, but several founder variants have been identified in individuals of different ancestries, including the BRCA2 c.156_157insAlu, in the Portuguese population. This variant accounts for up to one third of all hereditary breast cancer in the country. Previous studies were not concordant regarding BRCA BC prognosis. Our primary objective was to study the overall and cancer specific survival of BRCA2-P associated BC when compared to sporadic BC.

Two hundred and twenty four invasive BC patients were identified from 16 Portuguese Hospitals until June 2022. These pts had their BC diagnosed between September 1982-December 2021. Testing for c.156_157insAlu became routine practice for c.156_157insAlu after 2006. Cut off date for survival analysis was 31 December 2022.

One hundred ninety-eight pts (184 females and 14 males) were eligible for the study analysis. Median age of BC diagnosis was 44 yrs while the median age ate genetic testing was 50 yrs. From the 198 pts, 11 had bilateral BC at diagnosis and 187 unilateral BC. Within the latter group, 29 developed contralateral BC at least 6 months after the initial diagnosis. Clinical staging was as follows: stages I+II (145; 73%), stage III (48; 24%) and stage IV (5; 3%).

Most BC cases (178) were invasive carcinomas of no special type, 13 were lobular carcinomas, 6 had mixed pathology and 1 was tubular. As for immunohistochemistry 84,8% cases were HR positive while 9,6% and 7,1% were triple negative or Her2, respectively. With the exception of 5 pts all underwent surgery (97,5%). Chemotherapy, hormone and radiotherapy was prescribed in 86.3%, 84,3% and 71.2% of cases respectively. Uptake of risk reduction mastectomy (RRM) and salpingo-oophorectomy (RRSO) surgeries: 87 females consented on RRM (35 simultaneous with therapeutic mastectomy and 52 after a previous therapeutic surgery) and 121 consented on RRSO (117 after and 4 before BC diagnosis).

The median survival time for the BRCA2-P group was 20.85 years. When comparing to a control group from the National Cancer Registry (n=586) matched by age, gender, age of BC diagnosis and staging, no significant difference in overall and cancer-specific survival was observed. Multivariate analyses are being performed to evaluate the impact of modifier factors on BRCA2 c.156_157insAlu BC survival.

ASPIC Lecture – POPCARE: a nation-wide platform to speed up precision medicine in pediatric oncology

Author and Affiliation

Jorge Lima[1,2,3]

1. Ipatimup, Porto, Portugal

2.I3S, Porto, Portugal

3.FMUP, Porto, Portugal

Abstract

Introduction: The discovery of molecular biomarkers for pediatric oncology has facilitated important advancements in the diagnosis, risk stratification, and treatment approaches. Nevertheless, in Portugal, several challenges and barriers hinder the effective and widespread application of molecular biomarkers in pediatric oncology, creating inequalities in the access to these fundamental molecular tools.

Objectives: Our team proposes to address these unmet needs by creating a Precision Medicine Platform in Pediatric Oncology, known as POPCARE. This platform will foster collaboration between organizations from various sectors, including clinical centers, laboratory centers, the pharmaceutical/technological industry, and non-governmental organizations. The primary goal of the POPCARE platform is to facilitate the use innovative therapies in pediatric oncology.

Design/Methods: POPCARE will be implemented by performing a comprehensive genomic profiling of pediatric tumors, complemented by Molecular Tumor Board meetings, to optimize the processes of diagnosis, prognostic assessment, and therapeutic decisions. Furthermore, we will actively involve medical, scientific, and non-governmental organizations. They will ensure meaningful patient engagement, and play a vital role in the dissemination of information about POPCARE to patients and the wider public, providing accessible and reliable information about precision medicine, genomic profiling, and pediatric oncology. They will contribute to the structurization of genomic data, helping to establish standardized protocols for data collection, storage, and analysis, while also actively participating in the development of standards for genomic profiling in pediatric oncology.

Results: The POPCARE platform is currently in the implementation process, and will benefit from connecting with similar structures in Europe and from making itself known.

Conclusion: By actively involving medical, scientific, and non-governmental organizations, POPCARE aims to create a comprehensive and patient-centered approach to precision medicine in pediatric oncology, ensuring that the benefits of genomic profiling reach those who need it most.

USA-PT Lecture – Understanding & Taming Pancreas Cancer

Authors and Affiliations

Yonghong Liu[1], Jincheng Han[1], Wen-Hao Hsu[1], Kyle A. Labella[1], Pingna Deng[1], Xiaoying Shang[1], Paulino Tallón de Lara[1], Li Cai[1], Shan Jiang[1], **Ronald A. DePinho**[1]

1. Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030 USA

Abstract

In PDAC, oncogenic KRAS (KRAS*) drives glycolysis in cancer cells, leading to glucose depletion and massively elevated lactate levels within the TME. This metabolic landscape exerts a profound inhibitory effect on glycolysis-dependent CD8+ T cells and M1-like inflammatory TAMs while fosters a favorable environment for OXPHOS-dependent immune suppressive myeloid cells such as Tregs, MDSCs, and M2-like anti-inflammatory TAMs, thereby creating an immunosuppressive TME. Genetic or pharmacological inhibition of KRAS* yields profound tumor regressions across diverse PDAC models, partially attributed to the attenuated tumor-infiltrating MDSCs and M2-like TAMs and the augmented tumor-infiltrating T cells. However, the emergence of acquired resistance poses a substantial challenge, as evidenced by the inevitable relapse of tumors, highlighting the imperative of combining KRAS* inhibitors with adjunctive therapies for more durable responses in PDAC. Our previous studies have uncovered cancer cell-intrinsic and TME-mediated mechanisms of escape from KRAS* inhibition, providing actionable co-targeting strategies to enhance the effectiveness of KRAS* inhibitors. Here, we combined KRAS* inhibition with agents targeting the major arms of the immunity cycle: CXCR1/2 inhibitor for myeloid cells, antagonistic anti-LAG3 antibody for T cells, and agonistic anti-41BB antibody for dendritic cells. This combination elicited robust anti-tumor activity in iKPC mice bearing large autochthonous tumors. While vehicle/isotype-treated mice succumbed within 3 weeks, sustained treatment led to durable complete tumor regression and prolonged survival in 36% of mice at 6 months. Mechanistic analyses revealed enhanced T cell infiltration and activation, depletion of immunosuppressive myeloid cells, and increased antigen cross-presentation by dendritic cells within the tumor core. These findings highlight the promise of KRAS* inhibitors alongside immunotherapy as a potential PDAC treatment avenue, warranting clinical investigation

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Fluctuating methylation for lineage tracing in cancer

Author and Affiliation

Trevor A Graham

ICR, London, UK

Abstract

Cancers evolve, and the trajectory of evolution should determine prognosis or response to treatment. I will discuss how we have developed a new methodology, called EVOFLUx, that uses natural DNA methylation barcodes that fluctuate over time to quantitatively measure evolutionary trajectories using only a bulk tumour methylation profile as input. Applying EVOFLUx to large clinically-annotated cohorts of lymphoid malignancies reveals evolutionary trajectories are very powerful prognostic biomarkers, and provides new insights into clonal evolution and epimutation dynamics in these cancers. Widely-available, low-cost bulk DNA methylation data precisely measure clinically-relevant cancer evolution and provide an unexpected route to discover new cancer biology from old data.

SELECTED ORAL COMMUNICATION

Exploiting the Relevance of WNT6 in Extracellular Vesicles of Glioblastoma: Clinical and Molecular Insights

Authors and Affiliations

Helena M. Figueiredo[1,2], Eduarda P. Martins[1,2], Alexandra Teixeira[1,2,3], Lorena Diéguez[3], Paula Ludovico[1,2], Afonso M. Pinto[4], Marta Viana-Pereira[1,2,5], Bruno M. Costa[1,2,*], Céline S. Gonçalves[1,2,*]

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***** These authors contributed equally to the work

Abstract

Introduction: Glioblastoma (GBM) is the most common and lethal primary brain tumor in adults, partly due to technical challenges in diagnosis and monitoring. Liquid biopsies based on extracellular vesicles (EVs) offer a promising approach to overcome the existing limitations, as EVs are enriched in GBM patients' blood. Yet, EV-based biomarkers with true clinical value are still lacking. Our team previously linked high levels of intratumor WNT6, a WNT pathway activator, to increased aggressiveness and poor prognosis in GBM. Interestingly, WNT6 was later detected in high levels in GBM cell-derived EVs, raising the intriguing hypothesis that WNT6 in blood EVs may also have clinical relevance in GBM.

Materials and Methods: EVs were isolated from GBM and lower-grade glioma patient plasma samples and GBM cell lines genetically manipulated to express differential levels of WNT6, and were characterized based on the guidelines from the International Society for Extracellular Vesicles. WNT6 protein and mRNA expression levels in EVs and tumors were assessed through western blot and qPCR, respectively. RNA sequencing from WNT6+/- GBM cells and TCGA data from GBM patients was explored to assess associations between WNT6 and EV biogenesis.

Results: WNT6 cell transcriptomes revealed enrichment for several EV related processes, and TCGA data from GBM patients showed statistically significant correlations between WNT6 and EV biogenesis' genes. Higher EV levels were linked with increased WNT6 expression in both cell line-derived EVs and GBM patient EVs. Curiously, WNT6-high GBM patients presented a significantly higher level of plasma EVs than those with WNT6-low GBMs. Additionally, WNT6 expression levels in EVs were positively correlated with WNT6 expression in the respective glioma tumor.
Conclusion: WNT6 expression in GBM cells is associated with EV biogenesis, and WNT6 levels in GBM patients' blood derived EVs mirror its tumoral expression, highlighting its potential application for liquid biopsy approaches in GBM.

Leukemia Stem Cells: Seek and Destroy

Author and Affiliation

Alice Giustacchini

Human Technopole, Milan, Italy

Abstract

T-cells genetically engineered to express CD19 chimeric antigen receptors (CAR T-cells) have demonstrated remarkable efficacy in treating relapsed/refractory (r/r) B-cell malignancies, leading to their clinical approval for B-cell acute lymphoblastic leukemia (B-ALL) and Non-Hodgkin Lymphoma. Recent comparative studies of low-affinity and high-affinity CD19 CARs revealed that a second-generation low-affinity CAR, named CAT, significantly outperformed the high-affinity CAR used in Tisagenlecleucel (FMC63-based) in pre-clinical models. CAT exhibited enhanced expansion, cytotoxicity, and anti-tumor effectiveness, alongside an excellent safety profile, robust in vivo expansion, and persistence in a Phase I clinical trial. To understand the molecular basis for these superior properties, we conducted comprehensive in vitro analyses, comparing transcriptomic (RNA-seq) and proteomic (CyTOF) profiles of T-cells engineered with low-affinity versus high-affinity CD19 CARs after CD19 exposure. CAT CAR T-cells displayed a markedly stronger response to CD19 stimulation, characterized by distinct transcriptional and protein patterns, enhanced activation, and increased cytokine polyfunctionality compared to FMC63 CAR T-cells. This improved functionality is likely due to antigen-dependent priming by residual CD19-positive B-cells during production.

Considering the significant heterogeneity in Acute Myeloid Leukemia (AML) and the observed limitations of single-target CAR T therapies in this context, it is crucial to pursue strategies that target multiple antigens. Consequently, our current research focuses on employing single-cell multiomics to 1) systematically analyze the expression of surface antigens on pediatric AML stem cells (AML-SCs) and 2) develop and characterize multi-targeting CAR T cell products. This approach aims to design effective combinations of CAR T cells that can address the diversity and complexity of AML.

SELECTED ORAL COMMUNICATION

Sphingosine kinase activity is essential for IL-7R-mediated signaling in acute lymphoblastic leukemia, but not in healthy lymphocytes

Authors and Affiliations

Marta B. Fernandes[1], Alice Melão[1], Tatiana Araújo[1], Ana Cachucho[1], David R. Adams[2], Jeongah Oh[3], Afonso R. M. Almeida[1], Federico Torta[3], Vasco Barreto[4], Susan Pyne[2], Timothy Hla[5], Nigel J. Pyne[2], João T. Barata[1]

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5. Vascular Biology Program, Boston Children's Hospital and Department of Surgery, Harvard Medical School, Boston, USA.

Abstract

Introduction: IL7/IL7R signaling contributes to acute lymphoblastic leukemia (ALL) development. Sphingosine kinase (SK1/2) activity promotes cell survival. We aimed to dissect whether and how SKs crosstalk with IL7R-mediated signaling in lymphoid development and leukemia progression.

Materials and Methods: We generated mouse models to study SK1/2 deletion exclusively within the lymphoid compartment during normal (CD2Cre.SK1fl/fl.SK2-/-) and leukemic development (CD2Cre.Il7rcpt/wt.SK1fl/fl.SK2-/-). Cellular models included IL7-reliant ALL cell lines, primary or patient-derived xenograft (PDX) samples and healthy thymocytes. Cells were incubated with IL7 and SK pharmacological inhibitors. Flow cytometry analysis of cell viability, immunophenotype and signaling, co-immunoprecipitation, immunoblotting, CRISPR-Cas9 SK editing in HPB-ALL cells, transfection of HeLa cells, were used to characterize the impact of SKs on IL7-reliant ALL cells.

Results: CD2Cre.SK1fl/fl.SK2-/- mice showed no significant disruption of lymphoid development. In contrast, SK deletion delayed IL7R mutant driven B-ALL development and decreased STAT5 activation in leukemia cells. SK deletion in HPB-ALL cells prevented IL7-induced STAT5 activation and reduced cell viability. SK inhibition also blocked IL7-triggered viability in PDX T-ALL cells, without affecting healthy thymocytes. SK1 co-immunoprecipitated with IL7Rα in ALL cells and SK1 overexpression upregulated IL7R-mediated signaling in HeLa cells.

Overexpression of SKs may explain the heightened dependence of IL7R-mediated signaling on SK activity in ALL cells. SK inhibition reversed IL7-dependent pro-survival effects in human ALL samples. In a phase 2-like preclinical trial with 7 distinct IL7-responsive T-ALL PDXs, SK inhibition significantly increased mouse survival.

Conclusions: SKs are key players in oncogenic, but not in physiological, IL7/IL7R-mediated signaling, highlighting the therapeutic potential of SK inhibitors for IL7R-dependent ALL."

SYMPOSIUM II –TUMOR METABOLISM & EPIGENETIC CONTROL

Nutrient signaling to mTOR at the crossroads of cancer and aging

Author and Affiliation

Alejo Efeyan

CNIO, Madrid, Spain

Abstract

Cellular nutrients activate the mechanistic target of rapamycin complex 1 (mTORC1) via the Rag family of GTPases. This cascade, evolutionarily conserved in all eukaryotes, is key to couple the availability of building blocks and energy to the execution of the energetically onerous processes: protein synthesis, transcription, lipid synthesis and proliferation. Under plentiful levels of amino acids and glucose, a heterodimeric complex formed by RagA and RagC binds mTORC1 and recruits it to the outer lysosomal surface, which is an essential step for allowing mTOR kinase activation in a growth-factor dependent manner. Through a cascade of nutrient sensors, adaptors, GAPs and GEFs, the presence of nutrients results in RagA loaded with GTP, and RagC loaded with GDP.

Mutations in components of the Rag GTPase pathway are puzzlingly low in human cancer, except for GDP-like, activating mutations in RagC in B-cell lymphomas. We have engineered the mouse genome to knock-in some of these mutations and found that full-body $RagC^{mut/+}$ mice have accelerated lymphomagenesis when bred to the follicular lymphoma prone strain VavP-Bcl2. Mechanistically, heterozygous RagC mutations confer only a mild increase in nutrient signaling to mTORC1 that results in anomalous B-cell activation upon antigen stimulation. Strikingly, cells are only permissive to a subtle increase in Rag GTPase signaling, while massive deregulation of the pathway is deleterious, consistently with the absence of mutations leading to overt activation of the pathway in human cancer. Our current work points to a nutrient signaling – lysosomal biogenesis axis as critical for lymphoma development.

Without a lymphoma-prone genetic background, RagC$^{mut/+}$ mice exhibit a striking reduction in the spontaneous tumorigenesis that occurs at old ages, and a shortened longevity with multiple features of premature aging. The accelerated aging of RagC$^{mut/+}$ is not driven by increased Rag GTPase signaling in bone marrow-derived cells, as reconstitution of the BM with RagC$^{mut/+}$ in wt mice does not result in compromised longevity, whereas the reciprocal reconstitution of wild-type cells in a RagC$^{mut/+}$ host does result in premature aging. However, acute control of myeloid inflammation in aged RagC-mutant mice reverts some of the premature aging features, and extended elimination of myeloid cells extends the longevity of mice with increased nutrient – Rag GTPase signaling. We provide the first genetic proof of increased nutrient signaling and mTORC1 driving aging in mammals and support a two-component model in which increased nutrient signaling drives autonomously parenchymal damage, and myeloid inflammation further precipitates organ deterioration and accelerated aging.

SELECTED ORAL COMMUNICATION

Lactate in the epigenetic landscape recoding: towards new therapeutic strategies in renal cell carcinoma

Authors and Affiliations

Vera Miranda-Gonçalves [1,2], Ana Lameirinhas [1], Beatriz Costa [1], Catarina Macedo-Silva [1], Ana Pires-Luís[1,3], Paula C. Dias [1,3], Verónica Ferreira [1,3], Rui Silva-Santos [1,3], Isaac Braga [1,4], Maria Alzamora [1,3], João Lobo [1,2,3], Rui Henrique [1,2,3], Carmen Jerónimo [1,2]

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Abstract

Introduction: Renal cell carcinomas (RCCs), particularly clear cell RCC (ccRCC) represent some of the most lethal urological cancers due to their propensity for dissemination and metastization. An in-depth understanding at the cellular and molecular level of ccRCC holds promise for unlocking novel therapeutic strategies aiming to reduce cancer-related deaths. Recently, metabolite fluctuations were reported to dictate cancer cells' epigenetic plasticity, with implications for cancer aggressiveness. Therefore, dissecting the relevance of the epigenetic-metabolic crosstalk in driving ccRCC aggressiveness and metastasis could unveil potential therapeutic targets, thus enhancing outcomes for ccRCC patients.

Material and Methods: A total of 200 ccRCC primary tumors and 25 normal kidney tissues from the IPO Porto's Biobank were used for monocarboxylate transporters (MCTs), VHL, HIF-1α, sirtuins (SIRTs) immunoexpression analysis. (CES IPO:372/2017). The lactate effects on epigenetic enzymes, metabolic reprogramming, and cell phenotype were evaluated in normal kidney and ccRCC cell lines. Additionally, cells were exposed to sirtuin and MCT inhibitors, to investigate their impact in ccRCC cells, both in vitro, and in vivo (CAM model assay).

Results: MCT1, MCT4, HIF-1 α expression was upregulated in ccRCC, whereas VHL was downregulated. Lactate was found to modulate histone deacetylases' expression, and specifically decreased SIRT1 and SIRT6 expression in ccRCC cell lines. Downregulation of SIRT1 and SIRT6 by lactate has been shown to enhance cell migration and invasion by inducing epithelial-mesenchymal transition (EMT) and triggering a metabolic reprogramming switch.

Conclusion: MCTs overexpression in ccRCC coupled with increased lactate levels, fosters tumor cell aggressiveness, and induces malignant-like features in normal cells by downregulating SIRTs.

Mutagenesis, lesion segregation, and cancer genome formation

Author and Affiliation

Duncan Odom

DKFZ, Heidelberg, Germany

Abstract

Cancers arise through the acquisition of oncogenic mutations and grow by clonal expansion. In my talk, I will discuss our discovery that mutagenic DNA lesions are often not resolved into a mutated DNA base pair within a single cell cycle. Instead, DNA lesions segregate, unrepaired, into daughter cells for multiple cell generations, resulting in the chromosome-scale phasing of subsequent mutations. We characterized this process in mutagen-induced mouse liver tumours and show that DNA replication across persisting lesions can produce multiple alternative alleles in successive cell divisions, thereby generating both multiallelic and combinatorial genetic diversity. The phasing of lesions enables accurate measurement of strand-biased repair processes, quantification of oncogenic selection and fine mapping of sister-chromatid-exchange events. This lesion segregation is a unifying property of exogenous mutagens, including UV light and chemotherapy agents in human cells and tumours, which has profound implications for the evolution and adaptation of cancer genomes.

SELECTED ORAL COMMUNICATION

Nano-immunotherapy combination to regulate immune profiling and tumor-associated stroma in pancreatic cancer

Authors and Affiliations

Liane IF Moura[1§], Jéssica Cordeiro[1], Immaculada Conejos-Sánchez[2], Ana I Matos[1], María J. Vicent[2§], Helena Florindo[1§]

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§ Corresponding authors

Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by abundant desmoplasia and an immunosuppressive tumor microenvironment. Branched polypeptides (BP) have advanced engineered complexity and unique structural properties to be used as drug delivery systems. BP activate immune cells to modulate the release of tumor associated antigens/adjuvants for tumor growth reduction. Lymphocyte activation gene 3 (LAG-3) promote T cell activation and antitumor responses. Inhibition of Focal adhesion kinase (FAK) reduced fibrosis and enhance immune surveillance. This work aims to evaluate in vivo immune anti-tumor effect induced by BP delivering PDAC-associated peptide antigens MHCI and II ([BP-COL MHCI/II]) in combination with immune checkpoint inhibitor LAG-3 and FAK inhibitor (FAKi).

Materials and Methods: BP were synthesized/conjugated with MHCI and MHCII-COL peptide ([BP-COL MHCI/II]). To evaluate the nanoconjugate effect on ortothopic PDAC tumor growth, KPC cells were implanted into the pancreas of C57BL/6 mice. At day 7, mice were immunized with two doses of PBS, free antigens/adjuvants (MHCI/MHCII COL antigens+Toll-like receptor ligands), or [BP-COL MHCI/II] mixed with adjuvants, combined with αLAG-3 and FAKi. Mice weight were followed regularly. On day 20, mice were sacrificed and tumors collected.The expression of functional markers of different subtypes of tumor-infiltrating immune cells was quantified by FACS.

Results: [BP-COL MHCI/II] presented a mean diameter of 22nm and a zeta potential of -28mV, with a MHCI and MHCII peptide loading efficiency of 20 and 13% (w/w) respectively. A stronger tumor reduction was observed on mice treated with [BP-COL MHCI/II] plus αLAG-3 and FAKi, compared with PBS.Combined nanoconjugate-treated groups modulated tumor-infiltrating immune cells, leading to the local anti-tumor immunity by effector cytotoxic immune cells.

Conclusions: Combination of [BP-COL MHCI/II] with αLAG-3 and FAKi constitutes promissing strategy to overcome tumor growth in PDAC.

ASEICA Lecture – Understanding and predicting prostate cancer aggressiveness through the deconstruction of cell signaling and metabolism

Author and Affiliation

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Abstract

The products of metabolic pathways serve unexpected purposes in the process of cancer cell growth and dissemination. Despite the better understanding of the signaling and metabolic events that contribute to cancer, the field has not yet clarified (i) how these events are coordinately elicited, and, importantly, (ii) what differential signaling and metabolic cues drive cancer initiation and metastasis.

In order to decipher metabolic drivers of cancer, we envisioned a study that integrates bioinformatics screening, genetic mouse modeling and integrative metabolomics. We based our studies on the interplay between the signaling and metabolism in prostate cancer. We will provide an integrated perspective of the means and regulation of the signaling-metabolic switch in this disease emerging upon cell-intrinsic and cell-extrinsic perturbations. Specifically, we will elaborate on the distinct use of fuel for growth and the identification of signaling and metabolic pathways that serve to sustain biological processes related to disease progression. We will present the advantages of public transcriptomics dataset analysis through pre-existing and dedicated web interfaces to unveil gene expression changes that exhibit prognostic potential.

EACR Lecture – The metabolic cross-talk between T cells and tumor cells in melanoma-derived metastasis

Author and Affiliation

Ilaria Elia

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Abstract

Activation of the endogenous immune system through immunotherapy has greatly improved cancer treatment. Drugs that induce T cell function lead to the specific targeting and killing of tumor cells. Yet, especially in metastatic sites, a significant subset of tumors evade the immune system. Indeed, the tumor microenvironment (TME) represents a hostile niche that inhibits the function of infiltrating T cells and transforms them into an exhausted state. Recently, metabolic reprogramming has emerged as a key hallmark of immune responses. To support their proliferation and survival, T cells use fuels to generate precursors required for macromolecular synthesis, energy, and pro-survival pathways. As hyperproliferative cancer cells possess an overactive metabolism, depletion of nutrients and accumulation of metabolic waste products might represent a major nutritional hurdle for infiltrating T cells. Our goal is to map the metabolic requirements of T cells within the TME, develop strategies to potentiate T cell metabolism within this hostile niche, and thus use metabolic therapy to reactivate exhausted T cells and drive their cytotoxic function in immunocompromised metastasis.

SYMPOSIUM III – IMMUNOTHERAPIES & MICROENVIRONMENT

Expanding the cancer immunotherapy toolbox

Author and Affiliation

Noel de Miranda

LUMC, Leiden, Netherlands

Abstract

Checkpoint blockade immunotherapies have dramatically transformed cancer treatment and underscored the vast potential of immunotherapeutic strategies. Despite this, the majority of cancer patients do not benefit from these interventions. Consequently, there is a pressing need to expand and improve cancer immunotherapies. While the capabilities of T cells in recognizing cancer antigens have not been fully tapped, delving deeper into natural anti-tumor responses could reveal novel immunotherapeutic options based on atypical immune responses against tumors. In this talk, I will provide an overview of our efforts to broaden immunotherapeutic approaches by targeting cancer antigens, harnessing atypical anti-tumor immune responses, and exploring unconventional models of tumor immunology.

SELECTED ORAL COMMUNICATION

Immunosurveillance shapes the tumor genetic landscape selecting against putative immunogenic mutations

Authors and Affiliations

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Abstract

Immunosurveillance plays a crucial role in the detection and elimination of early-stage, clinically silent tumors but cancerous cells frequently devise strategies to evade this surveillance mechanism, enabling their growth and progression into detectable tumors. Despite extensive research of both phenomena, the precise mechanisms underlying cancer cell recognition and evasion remain elusive.

To better understand the immunoediting effect in tumor development, we used a mouse model of intestinal carcinogenesis with replication error deficiency (RER+) in immunocompetent (IC) and immunodeficient (ID) hosts. WES was used to compare tumor genetic landscape and identify distinctive attributes that are either selected or excluded by the immune system. Mutation frequencies in those genes were determined in human RER+ intestinal tumor samples. Immunogenicity of mutation-derived peptides was measured by IFN γ production upon co-culture of splenocytes from peptide-vaccinated mice with peptide-loaded dendritic cells.

ID animals display higher intestinal tumor incidence and burden, and a shorter lifespan than IC hosts, illustrating active immunosurveillance in intestinal carcinogenesis. Tumors in ID mice display a higher mutation burden than those in IC animals, and mostly exhibit uniquely mutated genes, suggesting selective immune-mediated shaping of the genetic landscape of tumors. Private mutations in ID tumors are primarily frame-shift or non-sense, and in vitro assays proved some of the mutations as immunogenic. These occur in genes that are frequently mutated in human RER+ intestinal tumors.

Our examination of tumor genetic landscapes in both IC and ID mice uncovered specific genetic mutations that are influenced by the presence or absence of immunosurveillance. The genes we identified have the potential to be incorporated into the designs of multivalent vaccines for clinical evaluation. This gap presents a unique opportunity to refine and expand the scope of vaccine-based strategies against cancer.

Integrated multi-omic analysis of adaptive immunosurveillance in early and metastatic breast cancer

Author and Affiliation

Stephen Sammut

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Abstract

B and T cells are key components of the adaptive immune system and mediate anti-cancer immunity. The T cell landscape in cancer is well characterised, but the contribution of B cells to anticancer immunosurveillance remains less explored. We performed an integrative analysis of the B and T cell receptor repertoires in metastatic and early breast cancer. Using immune receptor, RNA and whole exome sequencing, we show that both B and T cell responses appear to co-evolve with the metastatic cancer genomes and mirror tumour mutational and neoantigen architecture. B cell clones associated with metastatic immunosurveillance and temporal persistence were more expanded and distinct from site-specific clones. B cell clonal immunosurveillance and temporal persistence are predictable from the clonal structure, with higher centrality BCRs more likely to be observed across multiple metastases or across time. This predictability was generalisable across other immune-mediated disorders. This work lays a foundation for prioritising antibody sequences for therapeutic targeting in cancer.

SELECTED ORAL COMMUNICATION

Exosomes define a local and systemic communication network in healthy pancreas and pancreatic ductal adenocarcinoma

Authors and Affiliations

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) cells secrete exosomes, yet the intricacies of their in vivo spatiotemporal distribution, their specific target cells and organs, and the consequential impact on these recipient entities remains unknown. In our work we asked the question with whom do exosomes originating from the healthy pancreas and pancreatic tumors communicate, and what messages do they convey?

Here we use the genetically engineered mouse model (ExoBow) to map exosomes from healthy and PDAC pancreas in vivo to determine their biological significance.

One of our key findings is that the frequency of a particular cell type does not determine the communication routes that take place. This observation suggests that intercellular communication in vivo is not random but rather occurs in a coordinated manner. We show that, within the PDAC microenvironment, cancer cells establish preferential communication routes with cancer associated fibroblasts and endothelial cells. The latter being a conserved event in the healthy pancreas. Inhibiting exosomes secretion in both scenarios enhances angiogenesis, underscoring their contribution to vascularization and to cancer. Inter-organ communication is significantly increased in PDAC and occurs with the kidneys, lungs and thymus.

The integrated analysis of the cargo of PDAC exosomes, combining both protein and RNA content, along with the changes in RNA expression in fibroblasts and endothelial cells following exposure to cancer exosomes, provides valuable insights into the mechanistic underpinnings of observed in vivo phenotypes. This approach underscores the direct and indirect influence of exosomes on target cells, ultimately resulting in their reprogramming and the remodeling of the tumor microenvironment.

In sum, we found that exosomes mediate an organized intra- and inter- pancreas communication network with modulatory effects in vivo.

The immune landscape of breast cancer during pregnancy

Author and Affiliation

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Abstract

Breast cancer is one of the most common malignancies diagnosed during pregnancy. Despite the recent achievements in the management of breast cancer during pregnancy also referred to as pregnancy-associated breast cancer (PrBC), this condition remains clinically challenging and requires complex clinical management. PrBC is substantially similar to early-onset breast cancer (EOBC, i.e., breast cancer occurring in young, non-pregnant women) from a clinicopathologic and genetic perspective. However, recent evidence suggests that at least subsets of PrBCs are characterized by biological signatures associated with immune tolerance. Moreover, studies have underscored similarities between the immune mechanisms that facilitate maternal-fetal and tumor-host immunoediting. A broad understanding of the immunobiology of PrBC might provide insights into its etiopathogenesis, identify peculiar immune-related signatures, and finally improve the clinical management of patients with PrBC. Recently, our group conducted a comprehensive analysis of the tumor immune microenvironment in a large cohort of PrBC cases. We used morphology, immunohistochemistry, and gene expression profiling (GEP) to compare these cases with a control group of EOBCs. We showed that PrBCs are characterized by distinctive immunological features as compared to EOBCs, including the density of tumor-infiltrating lymphocytes (TILs), the distribution of CD4+ and CD8+ TILs subpopulations, and PD-L1 expression. Moreover, GEP analysis revealed differentially expressed genes between PrBC and EOBC. These genes belong to diverse superfamilies (Cancer/Testis Antigen, Interferons/Cytokines, Chemokines, and Immunoglobulin) and their expression levels are modulated according to hormone receptor status, TILs density and subpopulation. Our findings highlight the molecular heterogeneity and distinctive immune profiles of PrBC compared to EOBC. Variations in immune signatures and TIL subpopulations further underscore the critical role of the immune microenvironment in PrBC pathology. The different expression of specific immune-related gene families suggests their potential as actionable biomarkers. Future research should explore the clinical implications of these gene expression patterns to refine personalized clinical management strategies for PrBCs.

SELECTED ORAL COMMUNICATION

The zAvatar-test forecasts patient's treatment outcome in colorectal cancer: a clinical study towards personalized medicine

Authors and Affiliations

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Abstract

Introduction: Cancer patients often undergo rounds of trial-and-error to find the most effective treatment because there is no test in the clinical practice for predicting therapy response.

Materials and Methods: We conducted a clinical study to validate the zebrafish patient-derived xenograft model (zAvatar) as a fast predictive platform for personalized treatment in colorectal cancer. zAvatars were generated with patient tumor cells, treated exactly with the same therapy as their corresponding patient and analyzed at single-cell resolution. By individually comparing the clinical responses of 55 patients with their zAvatar-test, we developed a decision tree model integrating tumor stage, zAvatar-apoptosis, and zAvatar-metastatic potential.

Results: This model accurately forecasted patient progression with 91% accuracy. Importantly, patients with a sensitive zAvatar-test exhibited longer progression-free survival compared to those with a resistant test.

Conclusions: We propose the zAvatar-test as a rapid approach to guide clinical decisions, optimizing treatment options and improving the survival of cancer patients

RANK pathway-driven therapeutic opportunities in breast cancer

Author and Affiliation

Sandra Casimiro

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Abstract

The receptor activator of NFkB (RANK) pathway, known for its key role in osteoclastogenesis, is an important mediator of mammary physiology and carcinogenesis, by promoting the expansion of mammary stem cells and luminal progenitors. Conversely, RANK inhibition prevents or attenuates mammary tumor initiation, decreases tumor and metastasis incidence, and enhances anti-tumor immunity. Therefore, RANK has emerged as a potential target for BC prevention and treatment. RANK is more frequently found in triple negative breast cancer, where it is associated with an aggressive phenotype and is an independent biomarker of poor prognosis in postmenopausal women. Over the past years, we have been investigating the role of RANK signaling in luminal breast cancer, as well as the therapeutic potential of RANK inhibition across the different sub-types of breast cancer. Our main findings and current investigation will be summarized, to illustrate why we believe RANK pathway inhibition deserves further investigation and what should be the next steps.

SELECTED ORAL COMMUNICATION

A 3D tumor-on-chip platform to study intravasation

Authors and Affiliations

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Abstract

Metastasis is the primary cause of death in cancer patients and occurs mainly through hematogenous dissemination. As such, one of the first steps of metastasis is intravasation, the entry of tumour cells (TCs) in circulation. Intravasation is, however, one of the poorest understood processes of the metastatic cascade, mainly due to the lack of adequate in vitro systems to mimic it. Particularly unknown is how blood flow shear stress affects intravasation. In this study, we present a new perfusable 3D cancer-on chip system to study breast cancer (BC) intravasation. To optimize the system, we tested the cell viability and migration capacity of BC cells on different extracellular matrices (ECM), with different cell culture media and in microfluidic devices made of PDMS.

These devices were comprised of adjacent channels encapsulating hydrogel matrices to create a 3D microenvironment for cell homing and migration towards a hollow chamber, reachable for media exchange and vessel formation. In parallel, we optimized the conditions to generate a vessel-on-chip structure using different ECM coatings and endothelial cells (ECs) plating techniques. Finally, we perfused the endothelial lined channels and evaluated vessel integrity using anti-VE-cadherin antibodies and confocal imaging. This system allows that (at least) 80% of TCs are viable and invade the ECM, migrating collectively and reaching the endothelia seven days after being seeded. We were able to image with high resolution the interaction of TCs with ECs during intravasation under static conditions and we will now image intravasation under vessel perfusion. Overall, here we present a more sophisticated, hierarchical, and micro-physiological system to study intravasation than the generally used. We believe that this will contribute to better understand intravasation and to the development of new predictive, preventive, and therapeutic strategies to stop metastasis.

01. Multi-omics analysis of TNBC organoids reveals an Endosomal Recycling Regulation landscape specific of invasive cells.

Authors and Affiliations

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Abstract

Introduction: Metastatic dissemination is the main cause of cancer-related mortality. Yet, the underlying mechanisms driving cell migration and invasion are still poorly understood. Previously, we have shown that the endosomal sorting/recycling (ESR) pathway drives migration and invasion of Triple Negative Breast Cancer (TNBC) cells, downstream of FER kinase. Here, we hypothesize that ESR would do so by controlling the spatial distribution and turnover of adhesion and signaling complexes. So, different cancer cell populations would exploit distinct ESR pathway machineries and/or mechanisms for their regulation.

Methods: To test our hypothesis, we put combine TNBC PDXOs, single-cell transcriptomics and (phospho)proteomics, electron and confocal microscopies.

Results: Using electron microscopy, we observed a striking increase in organelles associated with ESR in invasive TNBC PDXO cells, namely ESR tubules and Golgi apparatus. In agreement, we observed an enrichment of phospho-events associated with organelle organization in invasive PDXOs. Also, invasive PDXOs cells downregulate the expression ESR machinery associated with non-invasive behavior. To validate the requirement of a specific ESR phosphoproteomic landscape to drive invasion in TNBC cells, we are currently investigating SEC16A, a key regulator of secretory and recycling pathways, that is phosphorylated downstream of FER in MM231 cells and in invasive PDXOs cells.

Conclusions: Overall, our observations are consistent with a model in which phosphorylation of ESR regulators drives the formation of recycling structures, necessary to control the spatial distribution of adhesion molecules at the plasma membrane, and subsequently promote TNBC cell migration and invasion.

02. Histopathological profile of Prostate Cancer in Subsaariana Lusophone countries: Cape Verde and Mozambique

Authors and Affiliations

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Abstract

Introduction: African American men of African ancestry face a higher risk for being diagnosed with prostate cancer, and other hand, a debate whether the advance stages detected at diagnosis are due to a unique aggressive biology of the disease or a delay in treatment is still ongoing. For instance, in the Cape Verde, prostate cancer is the most prevalent neoplasm in males, contributes to 21.7 % of all cancer-related deaths, making it the first most common cause of cancer death in males. And other hand, Mozambique with 1556 new cancer cases per year with prostate cancer (PCa) being the second leading cause of cancer. Despite the importance of PCa in Cape Verde and Mozambique, there is a lack. Moreover studies have shown that distinguishing between indolent pathology and aggressive tumors using prostate specific antigen (PSA) levels alone is insuficiente. Here, we investigated the role of chronic inflammation in histological profile of prostate cancer, particularly in late stage disease, and will discuss their potential use as biomarkers in order to design rational, specific and sucessful therapeutic approaches.

Materials and Methods: A total of 16 Consenting participants with elevated PSA with age ranged from 18 to 70 years and prior histologically confirmed prostate cancer through prior biopsy were recruited for this study at our research between February 2022 and December 2023. After radical prostatectomy, samples received from pathology department of publics hospitals (Praia and Maputo) were fixed immediately in 10% Neutral Buffered Formalin solution and embedded in paraffin. For morphological examination, 3-µm sections were stained with Hematoxylin & Eosin (H&E) and immunohistochemistry (IHC) was performed using Ki67 – MIB-1 (Dako, California, USA; Ref. IR62661-2). Antigen heat-retrieval was performed in a PT module (Fisher Scientific, New Hampshire, USA) at 98°C for 20 minutes using Envision retrieval solution Low pH, followed by incubation with the primary antibody. EnVision Link horseradish peroxidase/DAB visualization system was used, and sections were then counterstained with Harris' Hematoxylin and mounted. Representative photomicrographs of H&E and IHC slides were taken using NanoZoomer SQ slide scanner (Hamamatsu Photonics, Hamamatsu City, Japan) and sent to the researcher for analysis.

The slides were evaluated for prostatic adenocarcinoma by expert as per standard clinical procedure of histological diagnosis using a Gleason Score grading.

Results and Discussion: Our preliminar data indicate the morphologic features of the adenocarcinoma probably is reflected in as poorly diferrenciated, anaplastic features, glandular metaplasia, stroma, acinar and cribriform architectures and in particular, distintly infiltation of immune cells (chronic prostatitis). For now, poorly differentiated tumors were positive for Ki-67 of acinar adenocarcinoma cases.

We expect to reveal the Prostate Cancer Histopathological profile in Cape Verde and Mozambique, to help to have well detailed national policies to be put in place to increase detection of preinvasive lesions in order to reduce the prevalence of prostate cancer. Funding (INCUBATOR PROJECT): Calouste Gulbenkian Foundation and FLa Caixa Foundation.

03. Reprogramming exhausted T cell glycosylation: a novel immunotherapeutic strategy in colorectal cancer treatment

Authors and Affiliations

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Abstract

Despite the clinical success of immunotherapy, only a minority of CRC patients benefit from this therapeutic modality, highlighting the urgent need to identify novel mechanisms underlying cancer immunoregulation. Glycans play a pivotal role in each pathophysiological step of malignant transformation. Recent evidence highlights the importance of glycans as immune checkpoints in cancer immunosurveillance and immunoediting (Silva&Fernandes, Cancer Immunol Res 2020). Moreover, glycans have been shown to play a key role in the regulation of T cell-mediated responses. Here, we investigated the impact of T cell glycosylation in CRC progression.

We use colorectal cancer mouse models and further an in vitro protocol to generate exhausted T cells through exposure to persistent antigen under hypoxia and evaluate T cell glycosylation profile. In addition, we used CRISPR-Cas9 to alter the glycoprofile of therapeutic T cells by deleting key glycosyltransferases and assessed T cell effector function, cytotoxicity, and metabolic profile in vitro. We also tested the anti-tumor efficacy of glycoengineered therapeutic T cells in MC38-OVA-bearing mice.

We observed that along tumor progression there is increased expression of N-glycans. Analysis of multiple glycosylation events revealed exhausted T cells carry a distinct glycosylation program, with altered expression patterns of key glycosyltransferases. These results suggest that the expression of N-glycans plays a role in defining T cell properties in the tumor microenvironment associated with effector-memory vs. exhausted programs. Deletion of selected glycosyltransferases improves metabolic and anti-tumor capacity of antigen-specific T cells, revealing more robust tumor control in glycoengineered T cells in MC38-OVA-bearing mice.

Taken together, these results suggest the key role of glycans in cancer immunity and T cell exhaustion, and strongly suggests glycoengineering may be a critical component for the development of novel cancer immunotherapies.

04. Pirfenidone improves the antitumor and reduces the toxic effect of Vinorelbine plus Carboplatin in non-small cell lung cancer

Authors and Affiliations

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Abstract

Introduction: Platinum-based chemotherapy is a keystone for the treatment of non-metastatic or advanced non-small cell lung cancer (NSCLC) without targetable alterations and/or low PD-L1 expression. Pirfenidone (PF), an antifibrotic drug, was shown to have antitumor potential. This work aimed to study the chemosensitizing effect of PF to Vinorelbine (VR) or VR plus carboplatin (CBP) treatment, in NSCLC pre-clinical models.

Materials and Methods: NSCLC cell lines (A549, NCI-H322, NCI-H460) were used to evaluate the effect of drug combinations consisting of PF and VR on: 1) cell growth (sulforhodamine B assay); 2) cell viability (trypan blue exclusion assay); 3) cell proliferation (BrdU incorporation); 4) cell cycle profile (flow cytometry; PI staining); and 5) cell death (flow cytometry; Annexin V FITC/PI staining). Effect of a drug combination consisting of PF, VR and CBP (triplet) on NSCLC cell growth was also evaluated. Effects on growth of non-tumorigenic cell lines (MCF-10A, MCF-12A) were assessed. Athymic nude mice xenografted with A549 cells were injected i.p. with the vehicle, PF, VR with CBP (doublet) or the triplet, once or twice a week for five weeks. Tissues were collected for histopathological analyses, and blood plasma for biochemical analysis.

Results: PF sensitized NSCLC cell lines to VR treatment, reducing cell growth, viability and proliferation, altering the cell cycle profile and increasing cell death. Moreover, PF sensitized NSCLC cell lines to VR plus CBP, and this triplet drug combination did not induce additional cytotoxicity against non-tumorigenic cells. Importantly, the triplet reduced A549 xenograft tumor growth and proliferation, and decreased vimentin and collagen tumor expression. Remarkably, the triplet drug combination exhibited a safer toxicological profile than the doublet, indicating a protective effect for pirfenidone.

Conclusions: Together, our pre-clinical data supports the possibility of repurposing PF in combination with VR, or VR plus CBP, for NSCLC perioperative treatment.

05. Collateral sensitizer effect of novel Isoquinolinequinone N-oxides against multidrug-resistant cancer cells

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Abstract

Introduction: Reversing multidrug resistance (MDR) has become one of the main challenges of cancer research. The development of collateral sensitizers, i.e. compounds capable of exploiting the upgraded defence mechanisms of MDR cells as weaknesses, has been proposed as a strategy to overcome MDR. The aim of our work was to investigate the potential of two novel Isoquinolinequinones (IQQ) N-oxides as collateral sensitizers.

Materials and Methods: The activity of these compounds was tested in pairs of counterpart drug-sensitive and MDR cell lines from non-small cell lung cancer (NSCLC) and colon cancer. The effect on cell growth, viability, proliferation and cell cycle profile was evaluated. The toxicity to non-tumor cells was studied in the MCF10A cell line. To study mechanisms behind a collateral sensitivity effect, the following was evaluated on the NSCLC pair of counterpart cell lines: drug efflux pumps activity, ROS production, disruption of the GSH/GSSG balance and expression of key proteins associated with metabolism and redox balance (previously found to be differentially expressed between the NCI-H460 and NCI-H460/R cells). The antitumor and anti-MDR activity of the compounds was further confirmed in 3D cell culture models, using the CellTiter Glo assay on NSCLC and colon cancer spheroids having sizes around 350 µm.

Results: Results showed that both compounds presented lower GI50 concentrations in the MDR cell lines, when compared to their sensitive counterpart cells. One of the compounds increased ROS production, disrupted the GSH balance and altered the expression of proteins associated with protection against oxidative stress, particularly in the MDR cells. In addition, both compounds exhibited collateral sensitivity effect on spheroids of the MDR cell lines.

Conclusion: The IQQ N-oxide framework is promising to be further explored in the context of developing novel compounds to circumvent MDR, particularly collateral sensitizers. Future work should aim to assess the compounds' toxicity in vivo.

06. Exploring extracellular vesicle-based liquid biopsies to identify biomarkers of response to cancer immunotherapy

Authors and Affiliations

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Abstract

Although Immunotherapy is considered a breakthrough in Oncology, not all patients benefit from this type of treatment, with some showing primary or acquired drug resistance. Thus, there is an urgent need for predictive biomarkers of Immunotherapy response. Extracellular vesicles (EVs) contain a wide range of molecules in their cargo and have emerged as a novel source of cancer biomarkers. Due to their stability in biofluids, EV-based liquid biopsies hold great promise as a minimally invasive method for cancer diagnosis or response surveillance purposes. This work aims to explore plasma EVs as a potential source of predictive biomarkers for treatment response to immune checkpoint inhibitors (ICIs) in solid tumours.

Platelet-poor plasma from patients with solid tumours has been collected at (1) Baseline and (2) 4±2 weeks after initiating anti-PD-1/PD-L1 treatment. EVs have been successfully isolated by Size-Exclusion Chromatography followed by Ultrafiltration. They were further characterised according to their size and concentration (Nanotracking analysis), morphology and integrity (Transmission Electronic Microscopy) and particular protein content (Immunoblotting), respecting the MISEV2023 guidelines.

Our preliminary results indicate that EVs were efficiently isolated from plasma, with minimal contamination by non-vesicular proteins. Interestingly, we observed alterations in size, concentration, and expression of EV protein markers following treatment with ICIs. Important proteins known to be involved in the regulation of PD-L1 expression (e.g. STAT-3, STAT-1, EGFR), as well as PD-L1 protein glycosylation patterns, have also been identified in the EVs' content. Further correlations with patients' clinical outcomes will be performed.

The comparison of protein levels in the EVs' cargo and correlation with clinical outcomes may add knowledge to mechanisms of response and resistance to ICIs and contribute to identifying biomarkers of drug response.

07. Overcoming multidrug resistance through the collateral sensitivity effect of DNA-damaging drugs in non-small cell lung cancer cells

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Abstract

Introduction: Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases. The standard therapeutic option includes chemotherapy (taxanes and platinum agents). However, multidrug resistance (MDR) severely limits treatment efficacy. Interestingly, some drugs show stronger antitumor effect on MDR cells than on sensitive counterparts, thus presenting collateral sensitivity. This work aimed to identify collateral sensitizer drugs and study their effect on newly established NSCLC MDR cells.

Material and Methods: Two MDR cell lines (A549-CDR1; A549-CDR2) were successfully established, by treating sensitive A549 cells with increasing paclitaxel concentrations. The MDR phenotype was confirmed by Sulforhodamine B assay and Rhodamine 123 accumulation assay. Protein expression was evaluated by Western Blot. Flow cytometry was used to measure cell death (Annexin V-FITC/PI assay) and ROS production. DNA damage of cells was assessed by Comet assay.

Results: MDR cells had increased expression of drug efflux pumps and increased efflux of P-gp substract drugs, when comparing to sensitive counterpart cells. Interestingly, both MDR cell lines were more sensitive to DNA-damaging drugs (cisplatin, carboplatin, cyclophosphamide), indicating that these drugs caused a collateral sensitivity effect on the MDR cell lines. Following carboplatin treatment, the established MDR cell lines presented more DNA damage, higher expression of a DNA damage marker and lower expression of DNA repair enzymes than sensitive counterpart (A549) cells. ROS production, autophagic markers and glutathione metabolism in these cells are currently under evaluation.

Conclusion: Overall, two MDR NSCLC cell lines were established, as relevant cellular models to study collateral sensitivity. We found that DNA-damaging drugs exerted a collateral sensitivity effect on these MDR cells. Understanding the mechanisms underlying the observed collateral sensitivity effect may contribute to identifying approaches to overcome MDR in NSCLC.

08. PSGL-1 as an immunotherapeutic target in lymphoma

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Abstract

Relapses are common in lymphomas and many are refractory to immune checkpoint inhibitors. Therefore, new targets are needed. Since P-selectin glycoprotein ligand-1 (PSGL-1) was found to be important in lymphoma development and dissemination and was described as an immune checkpoint protein, we aimed to evaluate the PSGL-1 targeting potential in lymphoma development and in anti-lymphoma T cell responses. Human lymphoma cell lines were used for in vitro and in vivo tests with a PSGL-1 mAb. Healthy donor T cells were cocultured with irradiated Raji lymphoma B-cell line. T-cell activation was assessed by flow cytometry detection of CD25, CD69 and ELISA detection of IL-2 and IFN-γ production. For in vivo studies, BALB/c mice were s.c. injected with A20 mouse B-cell lymphoma cell line. We found that PSGL-1 targeting induced lymphoma cell apoptosis in vitro and in vivo, although not in all lymphoma cell lines tested. Regarding T cell responses, upon healthy human T cell coculture with irradiated Raji cells, the blocking PSGL-1 mAb resulted in: i) boosted upregulation of CD69 and CD25 activation marker expression and IL-2 production in pre-activated T cells; ii) increased percentage of CD4+CD69+ T cells and increased IFN-γ and IL-2 production in in vitro exhausted T cells; iii) increased CD69 and CD25 expression in primed T cells. Culture of lymphoma patient cells and autologous T cells showed that anti-PSGL-1 enhanced T-cell activation (CD69+) in three out of six cases. Finally, treatment of A20 lymphoma-bearing mice with 4RA10 mouse PSGL-1 mAb reduced tumor growth and increased the percentage of tumor immune infiltrates with increased percentage of activated CD8+ tumor infiltrated T cells and a reduction of Treg populations. In conclusion, we demonstrate that a PSGL-1 antibody was able to induce lymphoma cell apoptosis and that PSGL-1 blockade enhanced human T cell activation against lymphoma cells. These findings support the notion that PSGL-1 is a promising target for future lymphoma immunotherapies.

09. β-glucan polymeric microparticles: innovating cancer immunotherapy through targeted antigen delivery

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Abstract

Introduction: Cancer immunotherapies have emerged as a leading treatment strategy, particularly the ones harnessing antigen-presenting cells' (APCs) ability to elicit Th1 immune responses and priming cytotoxic T lymphocytes (CTLs). Cancer vaccination involves delivering tumor antigens directly to APCs to activate antigen-specific immunity against cancer cells. Here, we exploit the immunostimulatory properties of β-glucan polymeric microparticles (GMP) as a cancer vaccine platform for antigen delivery to APCs and adjuvant activity. GMPs can encapsulate antigens and other molecules, facilitating targeted delivery and efficient antigen presentation.

Materials and Methods: GMPs derived from yeast were characterized, assessing physicochemical properties and protein encapsulation efficacy. After isolation and differentiation of human peripheral blood mononuclear cells from healthy donors, uptake studies were conducted in monocytes, dendritic cells, and macrophages. In a vaccination study, mice received two subcutaneous doses of our formulation containing a model antigen and a TLR7 agonist encapsulated within GMPs. Spleens were isolated from vaccinated mice, and antigen-specific cellular responses were evaluated after in vitro restimulation.

Results: GMPs were preferentially taken up by dendritic cells over macrophages, with a tendency to accumulate in monocytes over time. Multiplex cytokine analysis revealed an antigen-specific Th1 immune response profile, characterized by elevated IFN-γ. Additionally, CTL effector function was enhanced, as indicated by increased percentages of antigen-specific Granzyme-B-positive CD8+ T cells essential for tumor cell elimination.

Conclusions: Our findings demonstrate that our GMP-based formulation efficiently targeted human APCs, inducing a robust Th1 immune response and enhancing CTL effector function. These results underscore the potential of GMPs as a cancer vaccine platform for immunotherapy.

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10. Targeting LOXL2 in Breast Cancer: Novel triazole derivative inhibitor

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Abstract

Introduction: Lysyl oxidases (LOX) are crucial for the cross-linkage of elastin and collagen in the extracellular matrix. Notably, LOX-like 2 (LOXL2) plays a significant role in promoting cancer cell migration and metastasis in breast cancer (BC) [1,2]. Consequently, there is an urgent need to identify inhibitors targeting this enzyme.

Materials and Methods: To understand the potential binding modes of inhibitors with LOXL2, we conducted docking studies using compounds documented in the literature. This revealed that structures such as indoline-aminoalkylphenol groups could inhibit LOXL2. In-house available compounds with such structures were screened for their anti-LOXL2 activity using the Amplex Ultra Red technique [3]. A triazole compound, synthesized following Rimpiläinen et al.'s method [4] exhibited inhibitory activity against LOXL2. Subsequently, we evaluated the effects of this compound on human BC cells (MDA-MB-231) and normal-like cells (MCF10A), assessing cell viability through the MTT assay and investigating its influence on cell migration using various assays including wound healing, transwell, and single-cell tracking. Immunofluorescence was also performed to evaluate its impact on DNA damage.

Results: Our findings revealed cytotoxicity towards both cancer and normal-like cells. While the compound, at non-toxic concentrations, did not affect collective migration and slightly increased individual migration speed, it significantly reduced chemotaxis and chemoinvasion of MDA-MB-231 cells, suggesting potential therapeutic benefits in BC treatment.

Conclusions: This study introduces a novel LOXL2 inhibitor capable of modulating the motility and DNA damage of MDA-MB-231 cells, offering promise for further development towards breast cancer therapy.

11. Development of ELISA assays based on advanced materials on lab-on-chip platforms for the detection of oncologic protein biomarkers.

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Abstract

Introduction: The potential of microgels and miniaturized devices in targeted cancer therapies and diagnostic procedures is investigated in this work, with an emphasis on immunoglobulin identification—specifically, IgG identification. It emphasizes how cutting-edge fibre optic integration with small Lab-on-a-Chip (LoC) systems has revolutionized and accelerated scientific and medical diagnostics. This integration, which reduces sample sizes, expedites analysis, and enhances precision, is essential for developments in point-of-care testing, personalized healthcare, and environmental analysis.

Materials and Methods: In the experimental part, microgels were conjugated onto surfaces for a new ELISA application. The concentration of the microgels in the feeding solution was adjusted to manipulate the microgel density.

Results: The study shows enhanced targeting of cancer cells through conjugation of microgels with antibodies, improving treatment efficacy and safety. This strategy also uses ELISA techniques to identify biomarkers, with a focus on biomarkers such as CA 125 and CEA, to enable early and highly sensitive cancer detection. The study also explores the area of liquid biopsy techniques that are improved by these cutting-edge technologies. It provides biomolecular recognition that is quicker, more accurate, and less expensive than conventional IgG detection techniques.

Conclusions: This study highlights the potential for numerous point-of-care applications and marks a significant advancement in medical diagnostics. It also highlights the potential benefits of utilizing multiplex assay techniques in the future.

12. RANK-driven androgen receptor upregulation and resistance to CDK4/6 inhibitors in luminal breast cancer

Authors and Affiliations

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Abstract

Introduction: The receptor activator of nuclear factor kB (RANK) pathway is an important player in progesterone-driven breast tumorigenesis by inducing the expansion of mammary epithelial cells. Current standard-of-care therapy for metastatic luminal (ER+) breast cancer (BC) is the combination of endocrine therapy (ET) with CDK4/6 inhibitors (CDK4/6i). We have shown that in ER+/HER2- BC, RANK overexpression (OE) is associated with resistance to ET+CDK4/6i. RANK OE xenografts accumulated several molecular traits associated with resistance to CDK4/6i, including RANK-driven senescent-like state and androgen receptor (AR) upregulation. In this work, we investigated if targeting senescent cells or AR could sensitize CDK4/6i-resistant cells to therapy.

Materials and Methods: CDK4/6i sensitive (MCF-7) and resistant (MCF-7 OE and MCF-7 PalboR) ER+ BC cell lines were exposed to inhibitors of CDK4/6 (palbociclib), RANKL (OPG-Fc), AR (enzalutamide) or a senolytic agent (navitoclax), in monotherapy or combined, for seven days. Cell proliferation was quantified by Alamar blue or BrdU assay. Therapy-induced senescence, as a surrogate marker of treatment efficacy, was quantified by SA-β-Galactosidase assay. The expression of hormone receptors (ER, PR and AR), and senescence or proliferation-associated proteins was quantified by Western blot.

Results: CDK4/6i resistant cells (MCF-7 RANK OE and MCF-7 PalboR) were characterized by increased expression of RANK, ER and AR at baseline. Activation of RANK signalling by exogenous RANKL increased the number of MCF-7 OE Sa-β-Gal-positive cells, but not MCF-7 or PalboR. The efficacy of enzalutamide or navitoclax in combination with CDK4/6i confirms that AR signalling and senescence represent escape mechanisms to CDK4/6i.

Conclusions: Our data suggests that AR or cellular senescence can be a new therapeutic approach in CDK4/6i-resistant luminal BC, supporting future studies.

13. Effect of RANK pathway inhibition in CDK4/6i-induced senescence and EMT

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Abstract

Introduction: The receptor activator of nuclear factor kB (RANK) pathway regulates progesterone-induced breast carcinogenesis and is associated with breast cancer (BC) aggressiveness. RANK signaling is implicated in epithelial-to-mesenchymal transition (EMT) and stemness, promoting tumorigenesis and metastasis. Therefore, inhibiting RANK pathway can positively impact BC outcomes. Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) are used in the treatment of hormone-responsive BC and we have shown that RANK inhibition prevents resistance to these drugs. CDK4/6i induce cell senescence, which may have dual effects in tumor control by arresting cell proliferation but also enhancing migration via senescence-associated secretory phenotype (SASP). In this work, we investigated therapy-induced senescence and EMT in BC cells treated with CDK4/6i and RANK ligand inhibitors (RANKLi).

Methods: BC cell lines responsive to CDK4/6i+RANKLi, either ER+/HER2- or triple-negative BC (TNBC), were exposed to palbociclib (CDK4/6i) with or without RANKLi (OPG-Fc and RANK-Fc) for 7 days. To determine if SASP components could induce EMT, untreated cells were exposed for 24h to the conditioned medium (CM) collected from treated cells. Therapy-induced senescence was quantified by SA-β-Gal staining, and senescence-associated and EMT-associated proteins were quantified by Western blot. Cell migration and invasion were quantified by transwell assay.

Results: Therapy-induced senescence and EMT were characteristic of CDK4/6i-treated cells, independently of RANK pathway inhibition. The CM of palbociclib-treated cells induced a senescent-like state in untreated cells, which were also characterized by increased expression of EMT markers. However, this was not accompanied by increased cell migration.

Conclusion: CDK4/6i-induced senescence can promote EMT via SASP, which seems to be unaffected by RANK pathway inhibition. The impact of such effect on cell outcomes and long term therapy efficacy warrants further studies.

14. THE CAM - Hatching an In Vivo Assay for cancer research

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Abstract

The Chorioallantoic Membrane (CAM) assay utilizing chicken embryos is a widely adopted alternative in vivo approach for investigating various aspects of cancer biology, such as angiogenesis (its primary application), but also tumor progression, invasion, metastasis, cancer stem cell dynamics, and drug response and resistance [1,2].

The inherent immuno-incompetence, at early developmental stages, enables the embryo to accept cancer cells from diverse sources without eliciting rejection. The CAM is a highly vascularized extraembryonic membrane, creating a nutrient-rich environment valuable to the spontaneous proliferation of human cells. This property has been validated with numerous cancer cell lines, primary cell cultures, and patient-derived xenografts. The CAM's accessibility facilitates manipulation, examination, and quantification upon exposure to cancer cells. The three-dimensional environment of the CAM supports the growth and development of xenografted cells and tissues, faithfully recapitulating the histological and morphological features of the original tumors.

Following the 3Rs principles for animal experimentation, advocated by regulatory bodies, the CAM model serves as a bridge between in vitro experiments and conventional mouse-based in vivo studies. Recognized as an in vivo assay beyond the scope of animal experimentation law (DL 113/2013), the CAM assay obviates the need for specific animal experimentation authorization. Thus, it stands as a valuable alternative method, generating predictive in vivo data that correlate with outcomes observed in rodent models, thereby significantly reducing the necessity for rodent experimentation.

The CAM scientific platform exemplifies an i3S' dynamic and ethical approach to advance scientific exploration in cancer research.

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2. Fischer, D. et al., Cancers 15, 191 (2023)

15. Unravelling the ErbB2 Sugar Code in Gastric Cancer: Construction of a Glycoengineered Cell-based Library

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Abstract

Introduction: Gastric Cancer (GC) presents a high mortality rate and its poor prognosis is mainly due to disease detection in the advanced or metastatic stage. Patients require systemic conventional and targeted therapies and, for the latter, few options are available, one being the trastuzumab (TTZ) monoclonal antibody. GC patients with ErbB2 overexpression, a cell surface receptor that undergoes extensive glycosylation, are eligible to receive TTZ. However, the vast majority experiences molecular resistance. The aim of this project is to understand if the ErbB2 glycosylation profile tunes TTZ binding and, consequently, its therapeutic efficacy. To do that, we are establishing a glycoengineered cell-based library for the cell surface display of ErbB2 carrying well-defined glycosylation profiles (ErbB2 GlycoDisplay)

Materials and Methods: In vitro characterization of the ErbB2 expression and activation levels in a panel of GC cell lines and their glycosylation profile by Western blot (WB) and immunofluorescence (IF), to allow the selection of cell lines for subsequent CRISPR/Cas9-based precise glycogene editing. Selected Cell lines undergo an established pipeline of precise genome editing with previously validated gRNAs; isolation and validation of independent cell clones and, finally, in vitro validation of their glycosylation profile by WB and IF.

Results: Two gastric adenocarcinoma cell lines showing high ErbB2 expression and endogenous activation were selected (NCI-N87 and OE-19) for glycoengineering. Several genomically edited cell line clones were obtained depicting tailored glycosylation profiles, that is, the selective abrogation of pre-determined cancer-associated glycan antigens. Successfully edited glycogenes include ST6GAL1, ST3GAL3 and FUT3. K.O. cell lines depict absent expression of α2,6- and α2,3-sialylation and sialyl Lewis a (SLea), respectively, from the cell membrane.

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.

16. Towards tracing lipid-nanoparticle intracellular trafficking in cancer cells by confocal Raman microscopy

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Abstract

Lipid nanoparticles (LNPs) can enhance the efficacy of mRNA-based cancer therapy by increasing bioavailability upon preserving mRNA integrity and stability until they reach their intended targets. However, the success and safety of LNPs is largely dependent on their intracellular trafficking. Confocal Raman microscopy (CRM) offers insights into the unique spectral fingerprint of samples with subcellular resolution and can shed light on the cell uptake and transport of LNPs. In this preliminary study, we investigate the potential of this imaging modality to track LNPs in live cancer cells.

For image acquisition murine mesothelioma (MM) AB1 cells were seeded into glass-bottom dishes (Ibidi, Wisconsin, USA) in a phenol-red free cell medium. LNPs encapsulating mRNA were produced by microfluidics using the Nanoassemblr™ (Precision NanoSystems, Vancouver, Canada). The physical properties of the LNPs were evaluated by dynamic light scattering (DLS). CRM of individual cells, LNPs, and LNPs components were performed using the LabRAM HR Evolution Raman confocal microscope (Horiba, Fukuoka, Japan). Sample excitation was accomplished using a laser emitting at a wavelength of 532 nm using an excitation power of about 4 mW. The integration time and number of accumulations were 10 s and 10, respectively.

We were able to image the assembled LNPs and distinguished Raman bands associated with individual components within their complex vibrational fingerprint. In addition, we successfully imaged label-free live MM AB1 cells, revealing Raman bands indicative of essential biopolymers present in cells, including proteins, lipids, and nucleic acids.

We have demonstrated the potential of CRM to detect and identify different cellular components and LNPs. In the future, we will further develop this method to image and track the dynamic incorporation process of LNPs into tumor cells.

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17. Il-6 induces the overexpression of genes involved in the regulation of apoptosis and cell-cell adhesion in polarized colorectal cancer cells

Authors and Affiliations

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Abstract

An inflammatory microenvironment was identified as a critical tumor-promoting condition for cells harboring tumor-initiating mutations. Cancer cells respond to pro-inflammatory signals with changes in their transcriptome, namely upregulating the expression of pro-tumorigenic transcript variants. A paradigmatic example is the variant RAC1B. We found increased RAC1B levels in samples from inflammatory bowel disease (IBD) patients or following experimentally-induced acute colitis in mouse models and showed it to be overexpressed in colorectal tumors. Recently, we found that the overexpression of RAC1B in polarized Caco-2 colorectal cancer (CRC) cells was triggered by the presence of pro-inflammatory interleukin (IL)-6. Here we describe the use of RNA-seq to characterize the transcriptome-wide changes induced in CRC cells by exposure to IL-6.

Total RNA isolated from control and IL-6-stimulated cells was subjected to library preparation and paired-end sequencing. Differentially expressed genes (DEGs) were identified by analyzing the counts per gene comparing control and IL-6 stimulation conditions and analyzed for biological process enrichments by Gene Ontology. Selected DEGs in significantly enriched categories were validated by semiquantitative (sq)RT-PCR.

We show that the most significant IL-6-induced transcriptional changes were associated with the upregulation of a cluster of 18 genes, mostly associated with the regulation of apoptosis and CEACAM-mediated cell-cell adhesion. Using sqRT-PCR, we validated the upregulation of three of these genes in IL-6 treated polarized Caco-2 cells. Interestingly, we found their expression to be downregulated in primary tumors of the CRC TCGA dataset but upregulated in samples from IBD patients. These results suggests that the observed IL-6 induced effects could be associated with early-stage transcriptomic reprogramming events that predispose for CRC development.

This work was funded by Fundação para a Ciência e a Tecnologia (PTDC/BIA-MOL/28386/2017) and EstagiAP2022.

18. TRIB2 pseudokinase as a novel regulator of glioblastoma invasion and stemness

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GB) is the most prevalent and aggressive primary brain tumor, ranking among the deadliest forms of human cancer. Unfortunately, therapeutic options remain limited to surgery combined with radiotherapy and chemotherapy, resulting in a median survival of only 15 months. The main challenge lies in the multiple resistance mechanisms and the extensive tumor invasion into neighbouring regions. Central to this challenge are GB Stem Cells (GSCs), contributing to tumor initiation, progression, invasion and being the main responsible for therapeutic resistance and recurrence. Hence, unravelling the mechanisms underlying GB pathophysiology, and particularly GSC biology, is crucial to develop more effective strategies. Recent discoveries by our group and others have identified a new player in GB tumorigenesis: Tribbles homolog 2 (TRIB2).

Methods: We evaluated TRIB2 mRNA expression in GB human samples and its correlation with clinical outcomes using public data bases. We also analysed TRIB2 expression in GB cell lines and patient-derived stem-like cells. Additionally, we generated TRIB2 knock-out GB cells using CRISPR/Cas9 to assess the impact of TRIB2 expression on proliferation, cell migration and stemness.

Results: Our findings indicate that TRIB2 is significantly upregulated in GB tumors compared to healthy tissue or low-grade gliomas, and its expression correlates with unfavourable patient outcomes. Moreover, we demonstrated that TRIB2 expression is increased in GSCs and it is downregulated during their differentiation. Importantly, our data shows that eliminating TRIB2 significantly impairs GB cell migration, self-renewal capacity and the expression of stem cell markers, thereby reducing their tumorigenic features.

Conclusions: Our data highlight TRIB2 as a key driver of migration and stemness, consequently contributing to disease recurrence in GB patients. Therefore, our research establishes TRIB2 as a promising therapeutic target and predictive biomarker for GB patients.

19. Characterizing the role of the Circadian Molecular Clock in T-cell Acute Lymphoblastic Leukemia

Authors and Affiliations

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Abstract

Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is a common and highly aggressive childhood malignancy. Despite recent treatment advances, relapse/refractory disease and severe side-effects are still major hurdles. The Circadian Molecular Clock (CMC) is an evolutionary mechanism that regulates homeostasis through 24-hour oscillations of clock genes (Bmal, Clock, Per and Cry), controlling a plethora of physiological processes. Notably, circadian disruption has been implicated in several pathologies, including cancer. Here, we sought to understand de role of the CMC in T-ALL.

Materials and Methods: We generated and characterized a mouse strain with Bmal1 conditionally inactivated from the common lymphoid progenitor stage onwards. The bone marrow progenitors of mice that express (Bmal1wt) or do not express (Bmal1null) Bmal1 were transduced with activated NOTCH1 (a potent leukemogenesis inducer) and injected into lethally irradiated mice. Time-to-leukemia was monitored. We also compared RNA sequencing data from T-ALL patient-derived xenograft samples versus healthy human thymocytes and focused on the expression of CMC genes.

Results: Bmal1 inactivation did not affect normal lymphoid development, but accelerated leukemia development.

Conclusions: Our results indicate that altered circadian oscillations due to Bmal1 inactivation do not affect normal T-cell development but accelerate the progression of T-ALL. These findings are in line with our transcriptomic analyses indicating that T-ALL patients display low levels of Bmal1 as compared to healthy thymocytes.

20. Colorectal cancer spheroid models: exploring tumor microenvironment dynamics and response to treatment

Authors and Affiliations

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Abstract

Recently, there has been a surge in interest in the study of the tumor microenvironment (TME) owing to its pivotal role in dictating tumor growth, progression, and therapeutic outcome. Consequently, the creation of three-dimensional cancer models that replicate the intricate interplay within the TME, alongside the structural complexities of tumors, holds paramount significance in advancing cancer research.

Herein, we developed and characterized colorectal cancer (CRC) spheroids over time according to the cells' susceptibility or resistance to doxorubicin (Dox). We then evaluated the effect of the inclusion of fibroblasts (heterotypic spheroids) in their response to Dox. Spheroid characterization included the evaluation of growth, viability, and hypoxia. Monitoring of Dox internalization and the effects of Dox-mediated chemotherapy on spheroid structure and viability were also studied to assess response to chemotherapy.

Results show distinct features for Dox resistance and the presence of fibroblasts. Fibroblasts are capable of stabilizing the spheroid through the modulation of their growth, cell viability, and hypoxia levels. These cells seem to promote the formation of cell-cell and cell-extracellular matrix interactions, increasing the recapitulation potential of heterotypic spheroids as models for the study of TME and resistance to chemotherapy. Dox resistance induction is shown to influence the internalization of Dox, especially in homotypic spheroids. The drug resistance profile seems to influence cell viability and consequently the chemoresistance of those spheroids when exposed to Dox.

Altogether, these results highlight the relevance of novel advanced cell models, with an increased recapitulation of the in vivo interactions of tumor cells with the surrounding environment.

21. Novel 3D human cell models to decipher the high-grade glioma tumor microenvironment

Authors and Affiliations

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Abstract

High-grade gliomas (HGGs) are central nervous system (CNS) tumors characterized by immunosuppressive tumor microenvironments (TMEs) that drive disease progression and therapy resistance. The dynamic interplay between CNS cells (e.g., astrocytes, microglia) and glioma cancer stem cells (gCSCs) fosters extracellular matrix (ECM) remodeling and HGG invasion. Understanding HGG immunosuppression and invasion mechanisms is key to improving therapeutic effectiveness. This work aims to develop a human cell model in which the main cellular and molecular interactions within HGG TMEs are depicted.

We employed a CNS/HGG 3D co-culture approach in stirred-tank culture systems. As CNS component, we resourced to neurospheroids (NSoids) derived from human induced pluripotent stem cells, which are composed of functional neurons, astrocytes, and oligodendrocytes within their native ECM. As HGG component, we generated gCSC-enriched tumorspheres (SOX2+, CD44+) from adult and pediatric HGG cell lines (A172, JX6, SF7761). In co-cultures, CNS and HGG cells remained viable and retained their identity at the phenotypic and gene expression levels. Heterotypic aggregate fusion events and adhesion of individual CD44+ HGG cells to NSoids were observed. In parallel, we established lentiviral vector-based green fluorescent protein (GFP)-expressing HGG cell lines and are exploring them for live monitoring of HGG behavior in co-cultures. Ongoing work is focused on microglia incorporation and in-depth ECM characterization of the CNS/HGG 3D model. Overall, this work establishes a set of robust tools to study complex cellular and molecular interactions in HGG TMEs and assess their modulation by novel therapies.

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22. 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 cases and refractory/relapse disease 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 in vitro and leukemogenesis in vivo. Furthermore, 10% of T-ALL cases display IL-7Rα gain-of-function mutations. The broad expression of, and dependence on, IL-7R in T-ALL provide 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 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 on T-ALL cells (western blot); 2) impact on the viability and proliferation of T-ALL samples (FC analysis); 3) internalization kinetics (FC analysis); 4) capacity to induce Antibody-dependent cellular cytotoxicity (ADCC) and/or 5) Antibody-dependent cellular phagocytosis (ADCP).

Results: Four mAbs with high-affinity binding to hIL-7R were selected: FJB26, FJB29, FJB45 and FJB48. None of these mAbs blocked IL-7Rα-mediated signaling in T-ALL cell lines and patient-derived-xenograft (PDX) samples, as evidenced by the lack of impact on the phosphorylation of IL-7R signaling effectors and on IL-7-mediated viability and proliferation of T-ALL cells. However, they all showed ADCC potential with FBJ45 displaying the strongest effect. Moreover, FJB29, FJB45 and FJB48 exhibited ADCP potential with the latter inducing the strongest effect.

Conclusions: Of the 48 mAbs screened for high IL-7R binding, we selected four for functional in vitro analysis. Of these, at least one mAb (FBJ45) displayed high capacity to promote T-ALL ADCC. We are in the process of validating this mAb in vivo using T-ALL xenograft mouse models."

23. Study of the Impact of the IL-7R on Non-Small Cell Lung Cancer

Authors and Affiliations

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Abstract

Introduction: Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) comprises most lung cancer cases, and, despite advances in diagnosis and treatment, it remains a global health burden. Interleukin-7 (IL-7) and thymic stromal lymphopoietin (TSLP) are cytokines whose receptors are heterodimers of IL7Rα and either γc or TSLPR, respectively. Due to their roles in regulating immune responses and tumor-promoting functions, they have been implicated in tumorigenesis. IL-7 plays a pivotal role in the proliferation and differentiation of immune players, including T- and B-cells, although it can also promote cancer cell growth. TSLP, recognized for its role in allergies and lung inflammation, has recently emerged as a mediator of tumorigenesis. Nevertheless, the precise roles of IL-7 and TSLP in NSCLC remain unclear.

Methods: Expression of IL7, TSLP and their receptors was analyzed by flow cytometry. Viability, proliferation, migration, and invasion assays were conducted across a panel of NSCLC cell lines to test their response to IL-7 or TSLP.

Results: NSCLC cells expressed IL7Rα, TSLPR, IL 7 and TSLP, albeit in different combinations and extents. IL7 did not activate signaling or promote cell survival, likely due to the absence of γc in the IL7Rα+ NSCLC cells that we tested. In contrast, TSLP activated STAT signaling in IL7Rα+ TSLPR+ H1975 cells but not in H3255 or Calu-1 NSCLC cells, which are IL7Rα+ TSLPR-. Control, non-malignant lung epithelial BEAS2B cells did not respond to either IL7 or TSLP. Notably, TSLP stimulation promoted migration, invasion, and proliferation of H1975 lung cancer cells.

Conclusions: Our work suggests that TSLP may promote NSCLC expansion via paracrine (and potentially autocrine) stimulation of lung cancer cells. Studies on the impact of IL7 are warranted using NSCLC cells known to co-express IL7Rα and γc. We are also evaluating the overall impact of IL7Rα expression for lung cancer progression in an IL7R knock-in model of Kras-mediated lung tumorigenesis.

24. Effective gold nanoparticle-mediated gene silencing: A transition from 2D cell culture to 3D tumor spheroids

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Abstract

Three-dimensional (3D) cell cultures using tumor spheroids have been gaining popularity as research models to replicate the tumor microenvironment. Several types of nanoparticle-based vectors have been employed for the efficacious delivery of RNAi moieties for gene modulation. Gold nanoparticles (AuNP) pose as valuable delivery vehicles in 2D cultures, but the same processes in 3D spheroids have not been so deeply characterized. Spheroids present additional features such as diffusion gradients and cell interactions, which more closely resemble the in vivo conditions.

Herein, we characterized the silencing efficiency of an AuNP-conjugate in 2D and 3D cultures. This nanoconjugate was designed to target the c-MYC oncogene for specific silencing. We demonstrate via RT-qPCR, Western-blot, and ICP-AES assays, that upon transitioning from a 2D cell model to a 3D spheroid, an increase of circa 2.6 fold the ratio of AuNPs per cell should be considered to attain similar silencing efficiencies. Such insights advance the development of targeted gene therapies within intricate tissue-like contexts.

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25. Therapeutic Nucleic Acids delivery in 2D and 3D tumour spheroids enhanced by Gold Nanoparticles and Visible Light Irradiation via Mild Hyperthermia

Authors and Affiliations

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Abstract

3D spheroids have become pivotal for the assessment of gene silencing strategies in models that more closely resemble the real tumour dynamics of cell uptake and internalization. For this, improving the delivery of the silencing moieties is of critical relevance. Delivery of antisense oligonucleotides and small interfering RNA into cells can be easily achieved using gold nanoparticles (AuNPs). Due to their unique optical characteristics, AuNPs provide additional features, such as the possibility for spatial-temporal triggering of cell uptake through light irradiation.

Herein, we used low-intensity photoirradiation combined with AuNPs for mild photothermy to improve the cell uptake of antisense oligonucleotides. We demonstrate this strategy by silencing GFP in two different 2D cell lines. We then show the effective silencing of the proto-oncogene c-MYC in 3D spheroids derived from colorectal cell line. Using this approach, we achieve effective gene silencing without toxicity to cells.

Our findings offer an innovative transfection approach that might enable spatial-temporal control of gene modulation.

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26. Effect of the gut microbiome in tumor development and immune response in a mismatch repair-deficient colorectal cancer model

Authors and Affiliations

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Abstract

Colorectal cancer (CRC) is highly incident and deadly. About 15% of CRCs have deficient DNA mismatch repair leading to microsatellite instability (MSI). While MSI tumors hold promise for immunotherapy, response rates remain low. Recent evidence showed that the gut microbiome influences cancer development and predict response to immunotherapy. Therefore, our aim was to use CRC with MSI as a model to explore the interplay between the microbiome and cancer immune response.

We modulated the gut microbiome of an inducible mouse model, characterized by loss of both Msh2 alleles in Lrig1-expressing quiescent intestinal progenitor cells, resulting in CRC with a MSI phenotype. The gut microbiome was depleted with antibiotics followed by repopulation with Bifidobacterium species. 300 days post tumorigenesis induction mice were euthanized. Fecal samples were collected for gut microbiome analysis and intestinal tumor tissues were collected for immune profile characterization.

Msh2 mice inoculated with Bifidobacterium species had a lower tumor incidence (39%; n=18) in comparison with control animals (60%; n=20). The number of tumors per animal and the tumor size was also smaller in Bifidobacterium-inoculated mice. Transcriptomics analysis showed 261 differentially expressed genes between tumors of the two groups. Functional annotation clustering categorized these genes into two strong clusters which identified pathways associated with the innate and adaptive immune response. Cell-type enrichment analysis revealed a decline in the adaptive immune response, characterized by decreased granulocytes and macrophages, along with an increase of the innate immune response, characterized by increased B and T cells in the Bifidobacterium-inoculated mice.

Further experiments are ongoing to characterize the microbiome's role in tumor development and immune modulation, potentially guiding innovative therapeutic strategies for the microbiome-immune-cancer axis.

PV has a PhD scholarship from FCT (2020.06228.BD).

27. Understanding how tumor-derived extracellular vesicles promote tumor growth and metastases

Authors and Affiliations

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Abstract

Extracellular vesicles (EVs) are nanosized structures released by all cells, that can transfer biomolecules locally and systemically. EVs derived from tumor cells are known to promote tumor growth and metastases, by establishing pre-metastatic niches in different organs. EVs excreted by various types of tumor cells express interferon-γ receptor (IFNGR). Interferon-γ (IFNγ) signaling leads to tumor cell killing and immune cell activation. However, IFNγ also induces the expression of the immune checkpoint PD-L1, which prevents T cell responses. Here, we aim to investigate whether IFNGR+ EVs transport IFNγ to future metastatic sites, and whether this promotes the formation of an immunosuppressive pre-metastatic microenvironment.

EVs produced by mouse metastatic melanoma B16-F10 cells were analyzed for their IFNGR expression and binding to IFNγ via Western blot and ELISA. To assess whether IFNγ-bound EVs can reach the lungs (preferential site of melanoma metastases), labelled EVs in presence or absence of IFNγ, were intravenously (IV) injected in C57BL/6J mice. Fluorescence was detected using Odyssey DLx, 24h later. To confirm that melanoma EVs promote tumor growth and metastases, EVs were IV injected 3x/week, for 4 weeks. After, B16-F10 cells were subcutaneously injected, and tumor growth was followed for 16 days. Organs where melanoma often forms metastases were analyzed ex vivo for their presence.

We verified that EVs isolated B16-F10 cells express IFNGR, bind to IFNγ, and accumulate in the lungs. Importantly, we also showed that EVs enhance tumor growth and the development of metastases in the lungs, brain and bones. Next, we will compare the capability of IFNGR+ vs IFNGR- EVs to form an immunosuppressive pre-metastatic microenvironment in these organs.

This research has the potential to unveil a yet overlooked mechanism by which tumor EVs promote the formation of pre-metastatic niches, which may provide crucial insights to optimize current cancer therapies and ultimately improve patient outcomes.

28. Establishment and characterisation of a RET/PTC1 thyroid cancer-derived cell line (TPC-1) variant with resistance to lenvatinib

Authors and Affiliations

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Abstract

Introduction: Aggressive thyroid cancer (TC) comprises 10-15% of all TC cases, impacting survival. Lenvatinib effectively controls advanced TC tumour growth. The emergence of genetic alterations/deregulation on lenvatinib targets (e.g., RET, FGFR1, VEGFR2) and non-targets (e.g., EGFR), may influence its efficacy. Hence, cell models to study lenvatinib resistance are warranted. Aim: To develop and characterise a RET/PTC1 TC-cell line (TPC-1) variant with lenvatinib resistance.

Methodology: TPC-1 lenvatinib resistant (TPC-1-LR) cells were generated from TPC-1 cells via a continuous exposure to lenvatinib. Lenvatinib's IC50 and resistance index (RI) were calculated. TPC-1-LR mutational and chromosomal profiles were obtained using next-generation sequencing (NGS) and comparative genomic hybridization (CGH). FGFR1, VEGFR2 and EGFR expression was assessed by qRT-PCR. Cytoskeleton's changes were evaluated by immunofluorescence (IF) for actin and vimentin.

Results: Lenvatinib's IC50 in TPC-1-LR (29.28 µM) was higher than in TPC-1 (6.12 µM), reflecting a RI of 4.7-fold. CGH showed no differences in chromosomal alterations between the cell lines. NGS profiling of TPC-1-LR unveiled an acquired NRAS p.Ala146Thr, potentially contributing to lenvatinib resistance. Gene expression analysis in TPC-1-LR revealed significant decrease of FGFR1 and VEGFR2 ($p < 0.01$), alongside with a 1.55-fold increase of EGFR ($p < 0.01$), a possible off-target resistance mechanism, when compared to TPC-1. IF assays showed increased actinic protrusions and vimentin expression in TPC-1-LR, indicating higher aggressiveness.

Conclusions: A TC cell model (TPC-1-LR) variant was established, exhibiting concomitant RET/PTC1 and NRAS mutations, unreported in other TC cell lines, and displaying resistance to lenvatinib. TPC-1-LR appears to be more aggressive than TPC-1. Gene deregulation and the NRAS mutation may contribute to this phenotype. TPC-1-LR may be a useful tool to investigate drug resistance and novel therapeutic approaches."

29. Copper(II) complexes with 2,2′:6′,2″-terpyridine derivatives: biological activities from 2D and 3D tumor spheroids to in vivo models

Authors and Affiliations

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Abstract

Ranked as the third most prevalent transition metal within the human body, copper assumes a crucial function across several biological functions. Notably, numerous cancer types demonstrate heightened levels of copper accumulation, linking this phenomenon to copper's involvement in cell proliferation, angiogenesis, and consequently, tumorigenesis and cancer progression. The differences observed in the responses of tumor and normal cells to copper enable the exploration of novel copper-based anticancer agents. Regarding that, eight 2,2′:6′,2″-terpyridines, substituted at the 4′-position with different aromatic groups, were employed to enhance the antiproliferative potential of [Cu2Cl2(R-terpy)2](PF6)2.

The cytotoxic activity of Cu(II) complexes was evaluated in A2780 (ovarian carcinoma), HCT116 (colorectal carcinoma, CRC), HCT116DoxR (CRC resistant to doxorubicin) cell lines and normal dermal fibroblasts. Besides their cytotoxicity, it was also assed their capacity to increase intracellular ROS, to interfere with cell cycle progression leading to cell death, their metastatic and pro- or anti-angiogenic properties, and their ability to interact with proteins and DNA.

Cu(II) complexes showed higher selectivity for the CRC cell lines, particularly for CRC cell line resistant to doxorubicin. As expected, the IC50 values in 3D models increased when compared with the values obtained for 2D cultures, in line with the greater complexity of the cellular microenvironment in 3D structures and more closing resembling an in vivo tumour. Cu(II) complexes are able to induce ROS production and consequently leading to cell death by both autophagy and apoptosis. They also demonstrated cytostatic, anti-metastatic and anti-angiogenic properties without in vivo toxicity, and to be able to interact with BSA. Therefore, these four Cu(II) complexes revealed properties that make them relevant for further preclinical studies.

30. ETV6::JAK2 fusion promotes central nervous system invasion in B-cell acute lymphoblastic leukemia

Authors and Affiliations

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy and remains an important cause of mortality in both children and adults. A subgroup of B-cell precursor ALL (BCP-ALL) patients with bad prognosis carries specific genetic alterations, including JAK2 fusions or mutations and CDKN2A deletions. Disease relapse in these patients is often associated with central nervous system (CNS) invasion.

Here, we hypothesized that JAK2 fusions are a risk factor for development of aggressive CNS-infiltrating BCP-ALL.

To study the impact of constitutively active JAK2 kinase signaling in CNS involvement by B-ALL, we generated mice expressing an ETV6::JAK2 fusion in the lymphoid lineage, in a background deficient in Rag2 and Cdkn2a genes.

Indeed, in comparison to Rag2-/-;Cdkn2a-/- littermates, ETV6::JAK2;Rag2-/-;Cdkn2a-/- mice exhibited earlier onset of B-ALL and presented more frequent CNS invasion (manifested as paraparesis or paraplegia). Immunohistochemical analyses of diseased ETV6::JAK2;Rag2-/-;Cdkn2a-/- and Rag2-/-;Cdkn2a-/- mice revealed leptomeningeal invasion by B220+PAX5+ cells expressing phosphorylated STAT5, a JAK kinase substrate. In vitro treatment of primary leukemic cells with a JAK2 inhibitor significantly impaired the survival of ETV6::JAK2;Rag2-/-;Cdkn2a-/- cells but not Rag2-/-;Cdkn2a-/- cells. To assess the dynamics of CNS invasion by B-ALL, we infused i.v. CD45.2-expressing ETV6::JAK2;Rag2-/-;Cdkn2a-/- and Rag2-/-;Cdkn2a-/- cells in Rag2-/-;CD45.1+ recipients and detected leukemic cells in the cerebrospinal fluid (CSF) at different timepoints, via flow cytometry analysis. Ten days post-injection, despite the presence of leukemia in peripheral blood in both groups, we detected CD45.2+B220+ cells in CSF of recipients injected with ETV6::JAK2;Rag2-/-;Cdkn2a-/- cells but not Rag2-/-;Cdkn2a-/- recipients.

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

31. Exploring tumor-resident microbiota in breast cancer

Authors and Affiliations

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Abstract

Introduction: Breast cancer (BC) is the most prevalent cancer and leading cause of cancer-related deaths among women worldwide. The presence of microbiota in breast has been recently described; however, a microbial signature has not been established and its role in tumor progression and metastasis remains unclear. Our project aims to isolate, visualize and identify intratumoral microbiota in primary and metastatic BC settings, to further define its potential prognostic, predictive and therapeutic value.

Material and Methods: To isolate viable bacteria, we dissociated patient-derived primary and metastatic BC samples, and cultured them in enriched bacterial media. Visualization of bacteria in situ has been conducted with antibodies against bacteria cell wall components in BC samples. Additionally, despite the challenge of extracting low biomass microbial DNA from these samples, we successfully amplified 16S rRNA full-length and identified tumor-resident microbiota using qPCR with phyla-specific primers and sequencing of the 16S rRNA V4 region.

Results: Tissue dissociation and culturing protocol allowed the identification of 18 viable bacteria species, 6 times more than in environmental controls. All the identified genera, including species of Staphylococcus and Streptococcus, had already been previously described. Confocal microscopy suggested that these bacteria are intracellular in BC samples. Amplification of the 16S rRNA full-length showed significantly higher levels in BC compared to controls, although lower than human feces. Additionally, metastatic BC exhibited 4 times less amplification of 16S rRNA gene than primary BC, and a different microbial profile.

Conclusion: These findings suggest the presence of viable intracellular bacteria in primary and metastatic BC. Moreover, 16S rRNA gene data support that the metastatic organ influences the microbiota profile. Comparing primary and metastatic microbiota may contribute to defining organotropism, offering potential therapeutic insights.

32. Unveiling the molecular fingerprint of early gastric cancer in a Western population with intermediate risk

Authors and Affiliations

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Abstract

Introduction: The asymptomatic nature of precancerous lesions constitutes a major clinical challenge that precludes a timely detection of gastric cancer (GC) in curable stages. The identification of novel and accurate biomarkers holds the promise of a better risk assessment and citizens stratification towards early diagnosis. In this exploratory study, we proposed, for the first time, to explore the transcriptomic landscape of gastric lesions (GL) in a Western population.

Materials and Methods: RNA-Sequencing analysis was conducted on RNA extracted from 39 formalin-fixed paraffin-embedded (FFPE) samples: 20 “normal-appearing” mucosa controls and 19 GL, including LGD, HGD, and adenocarcinoma. Differential expression and functional enrichment analysis were performed using the DESeq2 and goprofiler2 R packages.

Results: A total of 1171 differentially expressed genes (DEGs) were identified in GL, 557 down and 614 upregulated. The DEGs were significantly enriched in biological pathways associated with cell cycle, translation, and biosynthetic and metabolic processes. The Cancer Genome Atlas (TCGA) stomach adenocarcinoma (STAD) cohort was used for validation and 1101 DEGs were observed in stage I Caucasian patients. Similarly to GL, these genes were mainly involved in cell cycle processes, but also cell proliferation and differentiation. Considering the top dysregulated genes, KIF14 and HJURP were upregulated in the GL and TCGA-STAD sets, whereas ATP4A and CHIA were downregulated in both. On the other hand, the EREG gene was upregulated in GL (logFC=3.7, P=0.03) but downregulated in stage I Caucasian patients (logFC=-1.9, P=0.01).

Conclusion: This preliminary study identified commonly DEGs in early GL and stage I GC, highlighting shared biological pathways, but also revealing distinct expression patterns. These findings could potentially pave the way for improving early GC screening.

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33. Tributyltin's obesogenic action disrupts prostate glycolytic and lipid metabolism, stimulating cell proliferation

Authors and Affiliations

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Abstract

Evidence has pointed out prostate cancer as a public health issue strongly linked to environmental influences. Although the influences are diverse, substances like endocrine-disrupting chemicals (EDCs) have been blamed for this liaison. Tributyltin (TBT) is an organotin used as a biocide. It belongs to the subset of EDCs that alter energy metabolism to benefit lipid storage, the so-called obesogens. Metabolic reprogramming is a well-known hallmark of cancer, sustaining cell survival and metastization, which raises curiosity about obesogens-induced alterations. Herein, we investigated the effect of TBT dysregulating the prostate epithelium to a cancer-like phenotype by analysing metabolic and proliferative effects. Four-month-old Wistar rats were exposed to TBT (50 µg/kg) by oral gavage (45 days). After treatment, animals and prostates were weighed, and blood was collected. Protein expression of target regulators of glycolytic and lipid metabolism, as well as cell proliferation pathways, was analysed by Western blot. The enzymatic activity of lactate dehydrogenase (LDH) was determined spectrophotometrically. Proliferation was assessed by Ki-67 immunohistochemistry. TBT increased animal and prostate weight with serum metabolic alterations. The glycolytic profile was enhanced in TBT-treated animals, as indicated by the higher expression levels of phosphofructokinase 1, LDH and monocarboxylate transporter 4, and increased LDH activity. Regarding lipid metabolism, TBT augmented the expression levels of the fatty acid transporter CD36, while decreasing carnitine palmitoyltransferase 1A. Despite unchanged acetyl-CoA carboxylase expression, TBT augmented the expression levels of fatty acid synthase. TBT increased Ki-67-positive cells with raised expression levels of p-AKT and androgen receptor. This study demonstrates that TBT induces a dysregulation of prostate cell metabolism accompanied by enhanced proliferative status, supporting its role as a driving force in prostate carcinogenesis.

34. Do tumor cell:endothelial cell interactions modulate ciliogenesis, invasion and metastasis formation?

Authors and Affiliations

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Abstract

Introduction: Metastasis account for 90% of cancer-related deaths, therefore finding ways of blocking metastasis is a major need in oncobiology. One of the first steps of the metastatic cascade is cancer cell crossing of endothelial monolayers at blood vessels. In order to succeed during this process, cancer cells must properly interact with endothelial cells and be able to adapt to the blood flow shear stress they encounter while crossing the blood vessel wall. We have recently hypothesized that both processes exert a selective pressure over cancer cells that favors its metastatic success in colonizing distant organs.

Materials and Methods: Here we are testing this hypothesis by analyzing different breast cancer cell lines with different metastatic capacities for the way they interact with endothelial cells when transmigrating, and for the presence of flow sensing primary cilia. This was done using time lapse and confocal microscopy, respectively.

Results: From this analysis we were able to distinguish two different ways by which cancer cells interact with endothelial cells that distinguish metastatic and non-metastatic breast cancer cell lines. While metastatic cancer cells generally form cell contacts and stay side by side with endothelial cells, non-metastatic cancer cells extend protrusions and mainly “repel” endothelial cells. We are also studying the role of primary cilia in this context, as flow sensing organelles.

Conclusions: Although preliminary, our data suggest that tumor cell:endothelial cell interactions and flow sensing molecules, may contribute to this metastatic potential of breast cancer cells."

35. Escherichia coli “bioactive metabolites” as modulators of human prostate cells survival, migration and metabolism

Authors and Affiliations

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Abstract

Introduction: The microbiome is a new dimension of cancer related to the development and progression of the disease. Prostate cancer (PCa) bacteriome has been characterized and a relationship was found with cell proliferation. However, the importance of bacteria and their bioactive metabolites targeting different cancer hallmarks remains unknown. This work investigates the effect of Escherichia coli “bioactive metabolites” in PCa cell survival, migration and metabolism using a conditioned medium (CM) experimental approach.

Materials and Methods: E. coli-CM was produced by E. coli fermentation till the selected culture optical density was reached. Non-neoplastic (PNT1A), and androgen-sensitive (22Rv1) and castration-resistant (DU145) PCa cell lines were cultured with E. coli-CM for 48 h. Proliferation (Ki-67 immunocytochemistry), apoptosis (Caspase-3-like activity) and cell migration (Transwell assays) were evaluated. Spectrophotometric analysis was used to determine glucose and fatty acid consumption, lactate production and the activity of lactate dehydrogenase (LDH).

Results: Culture with E. coli-CM decreased the proliferative capacity of 22Rv1 and DU145 cells, concomitantly with increased caspase-3-like activity. In PNT1A cells, an increased migratory capacity was observed, while in 22Rv1 the opposite effect was noticed. Augmented fatty acid consumption was observed in PNT1A and DU145 cells cultured with E. coli-CM, followed by increased lipid content (PNT1A). Also, glucose consumption and lactate production were increased in DU145 cells, despite decreased LDH activity.

Conclusions: The obtained findings indicate that E. coli “bioactive metabolites” may alter prostate cells survival, migration and metabolism, demonstrating the importance of bacteriome in prostate carcinogenesis.

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36. the role of ANXA4 in glioblastoma

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Abstract

Introduction: Glioblastoma (GB) is a highly aggressive brain tumor that accounts for 48% of all primary malignant tumors of the central nervous system. Despite the standard of care treatment, which combines surgery, radiotherapy and chemotherapy with temozolomide, GB patients have a poor prognosis with a median overall survival (OS) of 15 months. Therefore, there is an unmet medical need to better understand the mechanisms underlying GB progression and to identify novel therapeutic targets. To address this, our lab has previously analyzed by RNA sequencing a cohort of paired samples from primary and recurrent GB tumors to identify differentially expressed genes. We found that Annexin A4 (ANXA4) is overexpressed in recurrent GB tumors in comparison with primary samples. We aim to validate our candidate gene ANXA4 as a promising player in GB progression.

Materials and Methods: We used publicly available dataset from TCGA to analyze the clinical relevance of ANXA4 in GB. Additionally, we overexpressed the ANXA4 gene in the GB cell line U87 and evaluated the effect on cell proliferation and viability. We also intracranially injected these cells in immunocompromised (NSG) mice and studied their impact in on tumor growth and mice survival.

Results: The TCGA dataset analysis showed that recurrent GB has higher expression of ANXA4 when compared to primary GBs and is associated with reduced progression free survival and OS in GB patients. In vitro, we observed that overexpression of ANXA4 in U87 cells increased cell proliferation, without affecting cell viability. Finally, ANXA4-driven mouse xenografts of GB showed increased tumor growth and reduced survival.

Conclusions: Our data suggests that ANAX4 is associated with a worse prognosis in GB patients and has a relevant role in disease progression.

37. PLCy1: Tumor supressor or oncogene in triple negative breast cancer

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Abstract

Introduction: Triple-negative breast cancer (TNBC), which lack HER2 expression and hormonal receptors is a particular type of extremely aggressive breast cancer with a faster growth rate, higher risk of metastasis and recurrence. Interestingly, we found that phospholipase C gamma 1 (PLCy1) expression is a good prognostic marker in TNBC. However, PLCy1 is overall considered an oncogene involved in cancer development and progression. It is known that PLCγ1 activated by growth factors such as EGF, VEGF and FGF regulates crucial processes to metastasis like cell motility, migration, and cytoskeletal rearrangement. This phospholipase is a converging point of many molecular pathways that modulate a plethora of tumorigenesis processes, cross talking with PDK1, AKT, RAS/RAF/ERK, JAK/STAT and Src.

Methods: To unravel the effective role of PLCy1 in TNBC prognosis, MDA-MB-231, MDA-MB-468 and Hs578T cell lines were used. With a PLCy1 overexpression model we evaluated the capacity of this cells to form colonies, calculate doubling time and confirm cell viability levels in comparison to WT. Through BrdU assay we were able to compare cell proliferation rates between PLCγ1 KO, PLCγ1 OE cells and WT. Since PLCγ1 is a converging point of pathways, we used western blot as a method to detect specific proteins that play a role in tumour progression.

Results: In this study MDA-MB-231 PLCγ1 OE cells were found to be less capable of colony formation and proliferation and have a higher doubling time. PLCγ1 KO cells had an increased proliferation rate as well as a higher expression of known tumour promoters.

Conclusion: These results reveal that PLCγ1 may have a protective potential and be a determinator of good prognosis in TNBC, although its necessary to increase the sampling size. Nevertheless, more assays are required to better elucidate the role of this phospholipase.

38. Therapeutic resistance in Myeloproliferative neoplasms: death cues from the bone marrow microenvironment

Authors and Affiliations

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Abstract

Myeloproliferative neoplasms (MPN) are clonal myeloid malignancies that rely on JAK-STAT signaling. The introduction of JAK inhibitors in the clinical practice improved symptoms but failed to reduce tumor burden. Therapy resistance is frequent in myeloid malignancies and the bone marrow (BM) microenvironment provides a protective milieu for leukemic cells to thrive. Research from our own group demonstrated that this microenvironment protects MPN cells from JAK inhibition by activating PI3K-Akt and JNK/SAPK signalling pathways. However, the microenvironmental cues responsible for such protective effect remain elusive.

The MPN patient derived cell lines were cultured in vitro with HS-5 bone marrow cells within the different experimental conditions. Upon culture, cellular viability was determined, and gene expression was evaluated by qPCR.

Inflammation deregulation is associated with severe MPN, however, the neutralization of proinflammatory HS-5 secreted cytokines did not impact on the BM-mediated protection of MPN cells. Based on this, we decided to screen for modulators of BM-mediated protection in MPN. We identified the TNFRSF8 and the TNFRSF9 genes as potential regulators that encode for the CD30 the CD137 receptors, respectively. These genes belong the Tumor Necrosis Factor Receptor superfamily and are involved in tissue homeostasis. MPN cells upregulated the receptors expression in contact with the BM, and the inhibition of these receptors with neutralizing antibodies dampened the BM protective effect to JAK inhibition.

Our preliminary results identify the CD30 and CD137 receptors as novel regulators of BM-mediated protection in MPN. Currently, we are expanding our studies to understand their relevance and the molecular mechanisms associated.

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39. T-cell receptor induction of CCR7 chemokine receptor promotes leukemic T cell dissemination

Authors and Affiliations

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Abstract

Introduction: T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) involves mainly the bone marrow, blood, and lymphoid organs, but can disseminate to other organs, aggravating disease. It was previously reported by us and others that absence of T-cell receptor (TCR) expression in T-ALL/LBL mouse models led to reduced dissemination of leukemic cells from the thymus to peripheral lymphoid organs, most notably lymph nodes. We aimed to understand which molecules could be involved in this TCR-dependent property.

Material and Methods: Gene mRNA expression, flow cytometry immunofluorescence analysis were performed from leukemic cells from the ETV6-JAK2 fusion transgenic mice (EJ-Tg). EJ-Tg mice were bred with Rag2, Ccr7 and Nfkb2 knockout (KO) mice; and with TCR-HY transgenic mice. Leukemic cells were infused intravenously in recipient mice.

Results and Discussions: Infused ETV6::JAK2 transgenic (EJ-Tg) mouse leukemic T cells lacking TCR expression (i.e., EJ-Tg;Rag2-/-) colonized much less efficiently the lymph nodes and spleens of recipient mice than EJ-Tg leukemic cells expressing endogenous or transgenic HY-TCR. Interestingly EJ-Tg;Rag2-/- leukemic cells expressed reduced levels of the Ccr7 chemokine receptor, a T-cell migration mediator involved in both thymic egress and peripheral lymphoid organ homing. By stimulating human T-ALL cell lines with CD3 antibody or the PMA phorbol ester, we confirmed that CCR7 expression was induced by TCR signaling. To study the role of CCR7 in vivo, we bred EJ-Tg mice with Ccr7 KO mice. Similarly to EJ-Tg mice lacking TCR (i.e. Tcralpha-/- or Rag2-/-), EJ-Tg;Ccr7-/- mice presented significantly larger thymic lymphomas and reduced splenic and lymph nodal involvement than Ccr7-sufficient littermates. By breeding EJ-Tg mice with OT-I TCR transgene and Ccr7 KO, we observed that the absence of CCR7 in OT-I;EJ-Tg mice resulted in lower lymph node and spleen dissemination of leukemic cells. To verify whether leukemic cells expressing Ccr7 was involved in homing to lymphoid organs, Ccr7-expressing EJ-Tg leukemic cells were infused in control littermates or mice KO for the Nfkappab2 transcription factor, which express reduced levels of Ccl19 and Ccl21 chemokines, the Ccr7 ligands, in the lymph nodes. Infused leukemic cells colonized less efficiently the lymph nodes of Nfkappab2-deficient mice, with no differences in the spleen and liver.

Conclusion: We conclude that TCR signaling is associated with expression of proteins associated with leukemic dissemination to specific niches and that CCR7 is a potential mediator of that property.

40. Modulation of exosomes secretion for improved immunotherapy outcomes in pancreatic cancer

Authors and Affiliations

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Abstract

Pancreatic Ductal Adenocarcinoma (PDAC) has a dismal 5-year mortality rate exceeding 90%. The current limitations of effective treatments underscore the urgency to identify new therapeutic approaches. Enhancing tumor susceptibility to immunomodulatory therapies presents as a viable strategy to improve patient prognosis.

We impaired the secretion of cancer exosomes in PDAC by utilizing genetically engineered mouse models (GEMM). Specifically, we created a conditional knockout (KO) of the Rab27a gene in cancer cells, thereby disrupting the production of exosomes by cancer cells impairing their capacity to reprogram immune cells and modulating the immune response to the tumor.

Interestingly, Rab27a KO led to earlier tumor onset and significantly decreased overall survival, but only with an intact adaptive immune system. These tumors exhibited a dominant proinflammatory response, driven by angiogenesis and recruitment of MRP8+ pro-inflammatory myeloid cells, fostering a Th17-tumor-supportive microenvironment. Therapeutic intervention with either CD4+ T cell depletion or anti-IL17A treatments impaired tumor growth rescuing the Rab27a KO phenotype. Conversely, Rab27a KO mice treated with dexamethasone showed impaired tumor growth, a response not observed in Rab27a wild-type counterparts with any of the described treatments. Remarkably, injecting cancer exosomes into Rab27a KO tumors significantly reduced their volume.

This study reveals the crucial role of Rab27a in PDAC progression and pinpoints molecular changes that offer potential targets for novel treatments. These findings lay the groundwork for developing therapies that capitalize on tumor´s altered immunological landscape.

41. Development of a workflow to capture circulating tumor cells (CTCs) from liquid biopsies of metastatic breast cancer patients

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Abstract

Background: Metastases arise from a dynamic process involving the systemic dissemination of circulating tumor cells (CTCs) throughout the body. Numerous studies have demonstrated the clinical utility of enumerating CTCs in liquid biopsies from breast cancer patients as a diagnostic and prognostic marker for monitoring therapeutic response and tumor recurrence. With the recognition of the enhanced metastatic potential of CTC clusters compared to single CTCs, the significance of CTC biotechnology has been further underscored. Therefore, our goal was to establish a workflow for identify, isolate, enumerate, and characterize CTCs in liquid biopsies from metastatic breast cancer (MBC) patients (stage IV). Materials and Methods: Within the framework of the P.CCC (Porto Comprehensive Cancer Center)-Raquel Seruca consortium, blood samples were obtained from IPOP (IPOP ethics committee: CES 64/023). Each sample, totaling 7.5 mL, was collected in CellSave Preservative tubes (Veridex, LLC). CTC enrichment and enumeration were conducted using ferrofluid nanoparticles conjugated with EpCAM-targeting antibodies, as well as immunofluorescent staining reagents, such as anti-CK-PE (specific for epithelial cells), anti-CD45-APC (specific for leukocytes) and DAPI (specific for nuclei of both cell populations).

Results: Until now, we have analyzed 23 liquid biopsies from MBC patients and detected more than 5 CTCs/7.5 mL of blood in 5 (21%) samples. Notably, we successfully identified CTC clusters, as well as leukocytes and CTC-leukocyte clusters, in one liquid biopsy. Association with clinical characteristics of MBC patients are ongoing.

Conclusion: This protocol has significant implications for the early diagnosis of metastatic disease, therapy stratification, and predicting relapses, with the potential to monitor disease progression and provide insights into personalized cancer treatments.

42. Targeting CD5 to Augment TCR-Mediated Leukemic Cell Death in T-Cell Acute Lymphoblastic Leukemia

Authors and Affiliations

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Abstract

T cell acute lymphoblastic leukemia (T-ALL) arises from a T-cell development arrest in the thymus, leading to immature T cell accumulation and malignant cell dissemination. The T-cell receptor (TCR) is crucial for T cell development and function. Although over 50% of T-ALL patients express this receptor in the leukemic cell, its impact on the disease is not entirely clear. While robust activation of TCR in leukemic cells can induce programmed cell death, recent evidence suggests it may also promote leukemia development. TCR negative regulators, such as CD5, are upregulated upon activation of the receptor and act thus control T cell responses.

Given that TCR activation has an anti-leukemic potential, we here aim to investigate if interfering with negative regulators would facilitate TCR-mediated leukemic cell death. Since CD5 is frequently expressed in T-ALL, we aimed to study the impact of its inactivation by CRISPR/Cas9 gene editing. We used the Jurkat E6.1 and HPB-ALL T-ALL cell lines, which express surface CD5, TCR and CD3. In vitro overnight stimulation with increasing concentrations of anti-CD3/CD28 monoclonal antibodies (mAb) revealed sensitivity to TCR/CD3 stimulation, resulting in leukemic cell activation (CD69 upregulation) and loss of viability. The CD5 gene knockout in both cell lines led to greater cell death, despite similar activation levels. We further analyzed TCR downstream pathways, namely phosphorylation (p) of Erk2 and ZAP70 proteins. Hence, we compared the levels of pErk2 and pZAP70 in CD5-deficient and -sufficient cells upon short anti-CD3mAb stimulation. Consistent with previous results, higher levels of downstream phosphorylation were detected in the absence of CD5.

In conclusion, our results demonstrate that TCR-mediated leukemic cell death can be enhanced by interfering with its negative regulator CD5. Therefore, identifying additional TCR negative regulators for potential targeting alongside TCR activation represents a promising therapeutic approach for TCR-expressing leukemias."

43. Genes promoter methylation as biomarkers of response to cisplatin chemotherapy in bladder cancer

Authors and Affiliations

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Abstract

Introduction: Bladder cancer (BC) is the 9th most incident worldwide. 25-30% of all cases are muscle-invasive (MIBC) tumors, which are commonly treated with platinum-based agents, namely cisplatin. Nonetheless, a primary obstacle in the utilization of cisplatin is drug resistance, which is currently under intense investigation. Thus, the main goal of this work is to identify novel epigenetic DNA methylation-based biomarkers to aid in the selection of bladder patients for chemotherapy, enabling the prediction of their response to cisplatin therapy in advance.

Material and methods: Six genes (BRCA1, RASSF1A, IRF7, RBBP8, APEX2 and MGMT) were chosen based on in silico analysis of TCGA public data for BC. Relevant CpG sites were pinpointed for the design of quantitative methylation-specific PCR (qMSP) primers and probes, and qMSP parameters were optimized. Pre-chemotherapy MIBC patient tissue samples (n=15) were selected based on chemotherapy response (responders and non-responders). Tumor FFPE slides were used for DNA extraction. DNA underwent bisulfite treatment followed by qMSP analysis for all samples. Subsequently, the obtained results were analyzed using adequate statistical analysis software.

Results: RASSF1A methylation levels were significantly higher in non-responders when compared with responders (p=0.045). Furthermore, IRF7me levels were also higher in non-responders (p=0.073). On the other side, APEX2 levels were higher in responders, while no differences were found in RBBP8, BRCA1 and MGMT methylation levels between the two groups of MIBC patient tissue samples.

Conclusions: Although these findings are preliminary results, RASSF1A and IRF7 show promising potential as candidates for predicting cisplatin resistance in BC patients. We anticipate expanding our patient cohort to enhance the robustness of the results obtained."

44. THOR: Tert Hypermethylated Oncologic Region- a novel breast cancer biomarker for liquid biopsy

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Abstract

Introduction: Breast Cancer (BC) is the leading cause of cancer-related death amongst women worldwide.1 The lack of robust clinical biomarkers for early screening and disease prognosis is one contributing factor to this scenario.2,3 In most BC cases, limitless self-renewal is achieved through hTERT reactivation promoting cancer development.4–6 One mechanism for this reactivation is the hypermethylation of a specific region within the hTERT promoter, called TERT Hypermethylated Oncological Region (THOR).7,8 Liquid biopsy provides real-time information about tumor status, particularly through circulating tumor cell-free DNA analysis.9 We aim to identify BC-exclusive THOR methylation patterns for prospective use as potential biomarkers in liquid biopsies.

Materials and Methods: After bisulfite treatment, PCR amplification and MiSeq sequencing, the raw DNA methylation data of the THOR region from 59 BC samples, 60 healthy breast samples and 47 healthy blood samples, was processed through a multistep bioinformatic pipeline: data quality control and poor bases trimming, alignment, incomplete bisulfite conversion reads filtration, methylation extraction, and complete reads selection. Then, we identified and quantified the THOR methylation patterns present in the BC tissue, healthy breast tissue and healthy blood. We selected the THOR methylation patterns detected in the BC tissue but never detected in both healthy breast and healthy blood samples, thus considered BC specific. This analysis was performed in BASH and in R.

Results: Comparing BC and healthy breast tissues, we identified 21,382 THOR methylation patterns BC specific that were never detected in healthy breast tissue. After comparing with healthy blood samples, we found that 15,039 of the 21,382 THOR methylation patterns remain exclusive to BC, with 23 patterns prevalent in at least 50% of the BC patients analyzed.

Conclusions: Our findings reveal the potential for THOR methylation signatures to be used as sensitive and highly specific BC biomarkers.

45. Modulation of CITED2 in glioblastoma unveils prominent traits

Authors and Affiliations

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Abstract

Introduction: Glioblastomas (GBM) are the most common and aggressive malignant brain tumors, characterized by high infiltration of the brain, resistance to therapies and an overall dismal prognosis. These tumors are composed of a heterogeneous population, including more undifferentiated cells known as Glioblastoma Stem Cells (GSCs). Since GSCs are likely responsible for resistance to therapies and relapse, therapeutic approaches towards these cells could be more effective. Therefore, the understanding of the mechanisms of stemness in these cells is urgently needed to unveil targets for therapy.

CITED2 is a co-transcriptional modulator and a key pluripotency factor in embryonic and adult stem cells. Moreover, it was implicated in several types of cancer either with pro-tumorigenic or anti-tumorigenic roles, but a potential role in GBM was not reported previously. The objective of this study was to determine if CITED2 impacts the biology of GBM.

Materials and Methods: CITED2 mRNA and protein expression in GBM cell lines was determined by RT-qPCR and Western Blot, respectively. To determine the impact of CITED2 levels on GBM biology, cell lines with CITED2 overexpression and knockdown were prepared, and self-renewal, proliferation, migration, viability, and tumorigenesis were evaluated on these cells vs the respective controls.

Results: By performing functional assays, we found that CITED2 overexpression promoted cancer stem cell properties such as clonogenic potential and sphere formation, an indirect measure of tumorigenicity assessed in vitro. On the contrary, CITED2 knockdown reduced the clonogenic properties of GBM cell lines.

Conclusions: Altogether, our results suggest that CITED2 may contribute to the self-renewal and tumorigenic properties of GBM. Therefore, modulating CITED2 expression in this context may be an effective approach to interfere with the stemness properties of GSCs.

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46. P-cadherin expression is a biomarker of a hybrid EMT phenotype

Authors and Affiliations

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Abstract

Epithelial-to-mesenchymal transition (EMT), once seen as a binary process is now understood to involve intermediate states where cells exhibit both epithelial (E) and mesenchymal (M) traits crucial for stemness, collective invasion, and immune evasion. Our previous data has linked P-cadherin (P-cad) overexpression to poor prognosis and aggressive tumor behavior in breast cancer. Yet, its role as a biomarker of a hybrid phenotype and in immune evasion remains unclear. The MCF10A-ER-Src model was used, where treatment with TAM for 6h, 12h, 24h and 36h, induces EMT. Flow cytometry assessed the expression of E (E-cadherin and CD49f), M (CD44 and CD61), and hybrid markers (CD104 and P-cad). PDL1 expression was also evaluated as a mesenchymal and immune evasion marker and a Live/Dead kit determine cell viability. The CAM assay was used to study the impact of TAM treatment at different timepoints on tumor formation and size. Data analysis utilized FlowJo 10.5.3 and GraphPad Prism V8, with significance set at $p<0.05$.

TAM treatment induced E cells to acquire a clear M phenotype after 36h, evidenced by a significant decrease in E-cadherin expression and CD49f median fluorescence intensity (MFI), along with an increase in CD61 expression and CD44 MFI over time. Additionally, CD104 MFI was enriched in the early timepoints, with a significant decrease at 36h. Furthermore, both P-cad and PDL1 expression exhibited a significant increase over time following TAM treatment. Using the CAM assay to evaluate tumorigenesis with TAM-treated cells for 12h and 36h, tumor formation and size remain consistent across all conditions, yet hematoxylin and eosin staining revealed a M morphology in tumors from TAM-treated cells, contrasting with E clusters formation predominantly observed in tumors from EtOH-treated cells.

Our observations indicate that P-cad is a biomarker of a hybrid phenotype. Further, we will study if there is a functional relation between P-cad enrichment and the increased expression of PDL1 and a possible role on immune evasion.

47. Profile and signature of bladder cancer-derived extracellular vesicles

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Abstract

Introduction: Bladder cancer (BlCa) is the 9th most frequently diagnosed cancer worldwide. The highly complex and heterogeneous nature of this malignancy impairs the effectiveness of early diagnosis and treatment. Thus, new alternatives to improve BlCa detection and management are urgently needed. Extracellular vesicles (EVs) are lipidic nanostructures that, to an extent, mimic their cell of origin, showing potential as biomarkers. Hence, we aim to identify unique protein patterns in BlCa-EVs, using 6 cell lines that can be classified as non-muscle invasive (NMIBC) and muscle invasive (MIBC) BlCa, representing different molecular subtypes.

Material and Methods: Cells were grown for 72 hours in multiple 150 mm petri dishes with 20 mL of medium containing EV-depleted fetal bovine serum until reaching 80-90% confluency. 3-6 biological replicates were used per cell line. EVs were isolated by ultracentrifugation (100,000g, 1h10min). Size distribution and concentration were assessed by Nanoparticle tracking analysis (NTA), shape by transmission electron microscopy (TEM), and protein content by micro bicinchoninic acid (microBCA). Currently, we are analysing the proteomic profile obtained by liquid chromatography–mass spectrometry (LC-MS/MS).

Results: A full characterization of the BlCa-derived EVs was performed. Based on NTA results, secretion dynamics differ between cell lines. SW780, a luminal NMIBC cell line, secreted the smallest amount of particles to the medium, whereas J82, a basal MIBC cell line secreted the highest number of EVs. Moreover, microBCA indicated differences between cell lines regarding protein profile.

Conclusions: Different BlCa-EVs exhibit a differential secretion pattern. Thus, comprehending these variances can help in unravelling biological mechanisms and assessing whether EVs faithfully represent the protein signature of their cell of origin. This study has the potential to reveal BlCa EVs' subtype-specific signatures.

48. Correlation between GDF15 and co-expressed genes in different types of cancer: a bioinformatic approach

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Abstract

Introduction: Growth Differentiation Factor 15 (GDF15) is a cytokine belonging to the transforming growth factor beta family, found to be elevated in various metabolic diseases such as diabetes, obesity, and several cancer types, namely colon, lung, and ovarian carcinoma. However, its role on tumor development or growth is not fully elucidated.

Bioinformatics tools and public databases may serve as powerful approaches to explore the intricate relationship between GDF15 and cancer. These tools can aid identifying differentially expressed genes associated with GDF15, the signaling pathways linked to its expression, and their relationship with clinical outcomes.

Methods: To explore the potential role of GDF15 in different tumor types, the 10 most positively and negatively co-expressed genes related with GDF15 were identified from cBio Portal. Genes that were simultaneously expressed in at least 3 tumor types were selected, and whether the relationship between those genes and GDF15 was previously reported in cancer was checked in PubMed.

Results: From 31 different tumor types available from PanCancerAtlas, 13 genes were co-expressed with GDF15 across more than 3 different tumor types, indicating a consistent association. Most of the identified genes were implicated in intrinsic apoptotic, inflammatory and endoplasmic reticulum stress signaling pathways, highlighting a possible association of GDF15 with these cellular events. Upon literature review, 5 of the positively associated genes were not previously described linked with GDF15.

Conclusion: There are novel putative players in cancer, associated with GDF15 expression, that may be further explored to gain novel insights about their potential roles in tumor biology.

49. Silent but Significant: Uncovering the Passenger Role of Transcriptionally Silenced Mutations in Breast Cancer Drivers

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Abstract

Introduction: The conventional understanding of cancer suggests that a small number of key mutations drive tumor growth, while thousands of other mutations present are considered passengers, having little to no effect. This view, however, often overlooks the actual expression levels of these mutations, potentially misidentifying mutations that are not expressed as significant cancer drivers.

Materials and Methods: We analysed data from The Cancer Genome Atlas (TCGA) on breast cancer (BRCA), combining whole-exome sequencing with allele-specific expression analysis of heterozygous coding mutations. This approach resulted in a dataset comprising 5,151 events from 628 patients. We specifically looked for cases where only the wild-type allele was expressed, followed by analyzing the mutational landscape and the functions of the genes involved.

Results: Our analysis showed that 6.4% of all heterozygous mutations, excluding known imprinted genes, were mutations not expressed in BRCA, indicating a significant but potentially passive role. These mutations, often of low frequency and challenging to identify in small-sample studies, primarily include missense and synonymous types across various genes, including cancer drivers, oncogenes, BRCA stem cell signature genes, tumor suppressors, among others. Their impact, which varies by mutation type, affects crucial cellular functions like transmembrane receptor activity, transcriptional regulation, cytoskeleton organization, adhesion, and metabolism.

Conclusions: Mutations not transcribed and hence ""invisible"" to cells, here termed functional passengers, are prevalent in breast cancer, suggesting their expression is controlled genetically or epigenetically via mutations in upstream regulatory elements, imprinting, or epigenetic silencing. Further studies are needed to link these mutations to specific regulatory mechanisms. Recognizing these transcriptionally silent mutations highlights the need to include expression data in therapeutic decisions to avoid targeting mutations not expressed in cancer cells."

50. Revisiting the CHEK2 variant p.(Arg346Cys): reclassification after 15 years?

Authors and Affiliations

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Abstract

Introduction: the CHEK2 gene is associated with hereditary breast, ovarian and prostate cancer (HBOPC). Unlike BRCA1/2 and PALB2 genes, CHEK2 is a moderate risk gene. Indeed, its penetrance is incomplete and a high prevalence of variants is observed in healthy controls, making variant classification challenging. Since included in our HBOPC molecular panels, we have classified several variants as variants of unknown significance (VUS) because of conflicting studies or insufficient data to classify otherwise. One example is the CHEK2 p.(Arg346Cys). The objective of this study was to assess the significance of this variant by analyzing its frequency within our cohort, family phenotypes and reviewing available data.

Patients and methods: From all pts tested for the whole CHEK2 gene we identified a study group including pts who tested positive for p.(Arg346Cys). A negative control group included all CHEK2 wt pts and a positive control group included pts with pathogenic/ likely pathogenic CHEK2 variant. We evaluated gender, personal/ familial cancer history, and tumor features across the groups. Additionally, we reviewed studies and available data regarding p.(Arg346Cys).

Results: From our cohort, 4479/7686 (58%) index pts were tested for CHEK2 gene and 17/4479 (0.4%) were diagnosed with the p.(Arg346Cys) variant. Demographic, clinical, and breast cancer pathological characteristics of pts is ongoing. Preliminary results and data review suggest reclassification.

Conclusion: Variant classification significantly influences patient care. While risk reduction options are available according to personal and/or family history of cancer, predictive tests can only be offered to family members of CHEK2+ pts. Although several studies support a damaging effect of the p.(Arg346Cys) variant, caution is recommended when dealing with moderate risk genes. Taking into account risk factors rather than relying solely on the classical classification may be an approach to CHEK2 variants until specific guidelines are established.

51. Unraveling the interplay among bladder tumor microenvironment, epithelial-mesenchymal transition and cytokine epigenetic regulation

Authors and Affiliations

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Abstract

Introduction: Tumor microenvironment (TME) and epithelial-mesenchymal transition (EMT) are important in non-muscle invasive (NMIBC) to muscle-invasive (MIBC) bladder cancer (BlCa) progression. Epigenetic reprogramming has been associated with TME modulation, including cytokine regulation. Cytokines can trigger EMT in different tumors. Here, we aimed to study the interplay among cytokines and EMT in BlCa and to uncover epigenetic mechanisms behind cytokine expression.

Material and Methods: A Cytokine/Chemokine Array was done. TGFB1, CXCL12 and BMP6 levels were evaluated in an IPO-Porto cohort and in The Cancer Genome Atlas. Cytokine expression was assessed in BlCa TME by a single-cell database (Chen et al. 2020). Cytokine levels were depicted after Panobinostat, Decitabine and Trichostatin A treatment in primary bladder fibroblasts and BlCa cell lines. BlCa cells were treated with exogenous TGF-β1, BMP6 and CXCL12, and EMT-related genes were evaluated.

Results: Normal urothelium showed a significantly higher BMP6 and CXCL12 expression, compared to BlCa TME. However, CXCL12 was significantly increased in MIBCs, than in NMIBCs, and BMP6 was slightly higher in MIBCs comparing with high-grade NMIBCs. Also, CXCL12 and TGFB1 expression was higher in metastases than in MIBCs. In silico data revealed that fibroblasts express high TGFB1 and CXCL12 levels. BMP6 was mainly expressed by fibroblasts and endothelial cells, albeit at much lower levels. In fibroblasts, CXCL12 and BMP6 expression increased after Panobinostat, suggesting histone acetylation as an important regulatory mechanism. TGFB1 seems to be regulated by DNA promoter methylation and histone acetylation in BlCa cells. Upon TGF-β1 and BMP6 stimulation, BlCa cells showed high CDH2, SNAI1 and SNAI2 levels, while CXCL12 increased CTNNB1.

Conclusions: TGF-β1, BMP6 and CXCL12 were disclosed as important cytokines in BlCa. These cytokines were epigenetically regulated in fibroblasts and in BlCa cells and can modulate EMT-related molecules in bladder tumor cells.

52. The role of Golgi ion channel Transmembrane BAX Inhibitor-1 Motif-containing 4 (TMBIM4) in glioma cell invasion and oxidative damage

Authors and Affiliations

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Abstract

Glioblastoma (GB) stands out as the most aggressive form of infiltrative glioma, due to the high heterogeneity, the ability to invade adjacent tissues and poor prognosis for patient survival rates. The Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) protein family, comprises six highly conserved intracellular ion channels (1 to 6), with diverse functions spanning crucial hallmarks of cancer. Considering this, we explored the role of TMBIM4 in glioma progression.

TCGA, CGGA, Rembrandt, and Ivy GAP datasets were used to examine TMBIM4 mRNA expression in gliomas and its association with patient survival. Knockdown (KD) experiments targeting TMBIM4 were conducted using human GB U87 and U251 cells in 2D and 3D cultures and in a mouse orthotopic model to assess its impact on glioma cell biology.

Analysis of glioma gene expression revealed a significant upregulation of TMBIM4 and a strong correlation between expression and glioma grade. Increased expression of 4 (HR 2.2) is correlated with reduced patient survival. TMBIM4 is particularly overexpressed in hyperplastic blood vessels and microvascular proliferation regions of GB, thus suggesting a role in tumour invasion. In fact, TMBIM4 KD strongly inhibited 2D and 3D cell invasion without affecting collective cell migration, viability, or proliferation and greatly reduced in vivo glioma growth in a mouse orthotopic model. Additionally, reduced TMBIM4 expression increased lipid peroxidation and DNA damage without measurable differences in total intracellular ROS. The Cystathionine Beta-Synthase expression dysregulation in GB in vivo following TMBIM4 KD suggests an unexplored connection between TMBIM4, the reprogramming of cysteine synthesis, and oxidative damage.

These findings highlight the potential of TMBIM4 as a therapeutic target or biomarker for glioma progression.

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53. Harnessing large-scale and single-cell RNA-Seq to Unveil the Landscape of Myeloid Lectins in Immunosuppressive Breast Cancer

Authors and Affiliations

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Abstract

Breast Cancer (BC) subtypes with poor prognosis are associated with tumor microenvironments (TMEs) rich in myeloid immune cells, known to promote pro-tumorigenic immunosuppressive (IS) TMEs. Immunotherapies targeting immune suppression, typically cytokine signaling, have been proposed. Yet they faced challenges from signaling redundancy and compensatory mechanisms, hampering clinical success. Recent findings emphasize the role of myeloid lectins, immune-modulating carbohydrate receptors that recognize aberrant glycans on tumor cells, in sustaining IS TMEs. As such, lectins are proposed as potential targets for immunotherapies, but systematic characterization of their expression pattern remains underexplored.

In this work, we profiled the lectin landscape of the myeloid cell population of tumor tissue from BC patients, to identify potential targets to block myeloid-mediated immunosuppression.

We analyzed a bulk RNA-Seq dataset (3207 patients) 1 and classified samples as IS (44%) and Non IS, using a regression model based on a classifying gene panel 2. HER2+ and Triple Negative tumors were overrepresented in the IS group, with decreased overall survival. Differential expression analysis of a curated list of lectin genes (116, coding immune transmembrane C- & I-Type), identified 12 upregulated in the IS group. We explored single-cell RNA-Seq datasets to define the myeloid cell subsets with expression of the identified lectins. Validation at the protein level in patient samples is ongoing and the next steps include functional validation.

In sum, we deliver a comprehensive characterization of the lectin landscape of myeloid cells within IS TME in BC, which may contain potential targets to tackle IS, rendering tumors sensitive to other immunotherapies.

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1 Dalal, H. et al., Sci Rep 12, 4696 (2022)

2 Tekpli, X. et al., Nat Commun 10, 5499 (2019)

54. From prostate cancer primary tumor to bone metastasis: Unveiling prostate cancer progression through novel bone metastasis cell lines

Authors and Affiliations

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Abstract

Introduction: Prostate Cancer (PCa) is a global health concern, mainly due to bone metastasis, which contributes to its high morbidity and mortality rates. Current PCa research models fail to recapitulate the metastatic cascade, with a notable lack of match primary tumor and metastasis cell lines for research. We established two bone metastasis (BM)-derived cell lines, CP2-Bo1 and CP2-Bo2, originating from the parental CP2 cell line derived from a prostate tumor of C57BL/6 Ctnnb1pc(ex3)Δ/+Ptenpc+/- mouse. We conducted in vitro studies to compare the behavior of these cell lines and high-throughput proteomic profiling to elucidate molecular adaptations occurring in PCa cells during progression from primary to BM tumor.

Methods: Cellular morphology, proliferation, colony formation efficiency, spheroid formation, 2D and 3D invasive capacity, and resistance to anti-androgen therapies (IC50 for bicalutamide and enzalutamide) were compared between the three cell lines. Protein expression was analysed by LC-MS, followed by exploratory, differential expression, and enrichment analysis.

Results: Morphologically, BM-derived cell lines exhibited a more circular shape and smaller area than the parental cell line. BM-derived cells displayed higher proliferative rates and colony formation efficiency, were faster to form circular spheroids, more invasive, and had increased resistance to anti-androgen drugs than the parental cells.

Proteomic exploratory analysis revealed distinct protein expression patterns among the three cell lines, with CP2-Bo1 and CP2-Bo2, both derived from BM, clustering together. Enrichment analysis of the upregulated proteins in CP2-Bo2 cell line highlighted pathways associated with epithelial-to-mesenchymal transition, hypoxia, focal adhesion, metabolism, and proteoglycans.

Conclusion: These matched-progressive primary and BM cell lines represent a novel tool for PCa BM research, as well as for identifying therapeutic targets and novel biomarkers for advanced disease.

55. Establishment and characterization of colorectal cancer oxaliplatin-resistant tumoroids to study chemotherapy resistance

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Abstract

Drug resistance and disease relapse remain major hurdles in cancer treatment and little is known about their underlying regulatory networks which is especially relevant in highly recurrent tumours, such as colorectal cancer (CRC). Here, we establish clinically relevant models of chemoresistance that will be used to identify and validate specific targets mediating acquired resistance in CRC.

A chemotherapy/radiation naïve patient-derived CRC tumoroid line was subjected to the chemotherapeutic agent oxaliplatin following a continuous and gradual treatment schedule to induce chemoresistance. Two resistant lines were generated at different concentrations, IC35 and IC55, which show a 5,2- and 13-fold increase in IC50 value, respectively. Dose-response curves showed that oxaliplatin-resistant lines also exhibit increased resistance to SN-38, but not to 5-FU. Immunohistochemical analysis showed that resistant lines have a strong increase in nuclear β-catenin and elevated KI-67 expression, which was also detected by RT-qPCR. Furthermore, resistant organoids overexpress the ABCC2 transporter and have elevated efflux of rhodamine-123, a P-gp substrate, accompanied by a significant increase in lumen-containing organoids. Resistant lines showed a 2-fold increase in the percentage of early apoptotic cells compared to the parental line. Oxaliplatin treatment strongly potentiated apoptosis in the parental line as opposed to the resistant lines, in which only a slight increase in cells undergoing early but not late apoptosis was observed. Our work describes the generation of a novel therapy resistance model that will be employed to uncover key mediators of chemoresistance and new potential anti-cancer targets.

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56. Deciphering WNT6-driven mechanisms of aggressiveness and resistance to chemotherapy in human glioblastoma

Authors and Affiliations

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Abstract

Introduction: Glioblastoma (GBM) is a highly lethal cancer with limited treatment success. Thus, it is urgent to identify biomarkers predictive of GBM therapy response and elucidate their underlying mechanisms, potentially uncovering novel therapeutic opportunities. Our team demonstrated that WNT6, a WNT pathway activator, is an oncogenic molecule with prognostic value in GBM, yet its underlying molecular mechanisms are largely unknown.

Materials and Methods: The transcriptomes of matched GBM in vitro models genetically-engineered to express differential levels of WNT6 were defined by RNA-sequencing. The impact of WNT6 expression on the sensitivity to temozolomide (TMZ), the standard GBM chemotherapy, was explored through in vitro functional and molecular assays, and in vivo orthotopic experiments. RNA-sequencing data were validated and the associations of WNT6 with classical mechanisms of TMZ response were investigated in GBM patients' data. Finally, the effects of WNT6 expression in the clinical outcome of GBM patients treated with TMZ were studied.

Results: We uncovered novel enriched processes and pathways that further define the oncogenic roles of WNT6 in GBM, and identified enrichment of terms related to therapy response, supporting preliminary data suggesting WNT6 may have a role in TMZ response. Importantly, high WNT6 expression was associated with decreased TMZ effectiveness both in vitro and in vivo. Molecular assays and omics enrichment analyses unraveled putative mechanisms through which WNT6 could be reducing TMZ effectiveness, namely through effects in DNA repair mechanisms. Finally, high WNT6 expression was identified as an independent predictive biomarker for a decreased survival of chemotherapy-treated GBM patients.

Conclusions: This work suggests WNT6 is predictive of chemotherapy response in GBM, and sheds light into the molecular mechanisms through which WNT6 may be operating and therapeutically-targeted in future precision medicine-based approaches.

57. Tumor-targeting strategies for the development of selective therapies: p28 and drug penetration using in vitro 3D cancer spheroids

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Abstract

Introduction: Conventional systemic cancer therapies induce severe side-effects, which limit their efficacy and safety. In the last decade, extracellular vesicles (EVs) emerged as promising nano-drug delivery systems (NDDS). These display amenability for loading with multiple anticancer therapeutics and engineering aiming to improve their accumulation at the target tissues. The anti-cancer cell penetrating peptide p28 has recently been demonstrated to facilitate the uptake of nanoparticles in lung cancer and deliver efficiently anti-cancer drugs [1].

Materials and Methods: In order to develop an EV-based NDDS for targeted cancer therapy, mesenchymal stromal cell (MSC) derived-EVs were isolated through a GMP-compatible scalable protocol comprising tangential flow filtration (TFF) and size exclusion chromatography (SEC). EVs were decorated with the azurin-derived p28, a peptide displaying preferential penetration into cancer cells. Moreover, the ability of p28 to diffuse through breast cancer spheroids has been tested. MCF-7 spheroids were assembled using agarose molds casted on the 3D Petri Dish® micromolds (MicroTissues Inc., USA).

Results: Incubation of breast cancer cells with EVs-p28 led to an increased cell uptake of EVs by 1.4 - 2.4-fold in comparison to native EVs. Regarding the p28 penetration, spheroids were generated and fluorescently labeled p28 with 5(6)-carboxyfluorescein(FAM; 25 µM of labeled peptide over a total of 100 µM of p28) was added at Day 3 and the degree of penetrations was evaluated at Days 4 and 7 using confocal imaging. On Day 7, after 96h of exposition to p28, the peptide is present in the central areas of the spheroid.

Conclusion: Overall, this novel NDDS could be valuable for promoting increased tumor retention of anticancer agents that have associated off-target effects, rendering them more effective. We will now evaluate how the functionalization of EVs with p28 may impact their penetration through the spheroids and influence cells' drug resistance profile.

[1] Garizo AR, Castro F, et al. (2021) J Control Release (337):329-342. doi: 10.1016/j.jconrel.2021.07.035

58. Characterization of the immune profile of medulloblastoma subgroups: a transcriptomic approach

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Abstract

Introduction: Medulloblastoma (MB) is subdivided into 4 molecular subgroups: WNT, SHH, Group 3 and Group 4. Treatment involves surgical resection, radio- and chemotherapy, leading to serious adverse effects and non-response in high-risk patients. The application of immunotherapies could change this paradigm. However, current attempts with immune checkpoint blockers reported disappointing results. Recently, we found that MBs express high levels of alternative immune checkpoint genes, namely CD24 and CD276, with important prognostic values. Still, deeper immunological knowledge is needed for a rational use of immunotherapies.

Objectives: To characterize the immune expression profile of human MB, evaluating potential prognostic biomarkers and treatment targets.

Methods: The expression levels of 730 immune-related genes were evaluated in 88 FFPE MBs through nCounter applying the PanCancer Immune Profiling Panel (Nanostring). Immune profiling for each subgroup was made using the R software. Further validation was performed in two public microarray datasets (n=1350) and one single-cell RNA sequencing dataset (GSE155446).

Results: All subgroups showed high expression of anti-inflammatory cytokines and chemokines such as VEGFA, CSF1, TGFB1, TGFB2, CCL2, CCL3, CCL4, CXCL12 and its receptor CXCR4. Furthermore, we found genes that were more expressed in a specific subgroup and those differences were confirmed by the single-cell data, showing the genes EOMES (Group 4), ALCAM, MAF (WNT), TGFB2 (SHH) and VEGFA (Group 3) to be representative of specific subgroups. Both the microarray and single-cell data showed a preference for myeloid immune cells infiltration with an anti-inflammatory phenotype characterized by the expression of TGFB1, CXCR4, CD48 and HAVCR2.

Conclusions: Overall, our findings suggest new possible targets and that the MB immune microenvironment has a high presence of immune myeloid cells indicating possible routes for future immunotherapeutic approaches.

59. Targeting glucose metabolism in acute myeloid leukemia (AML) chemoresistance

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Abstract

Altered glycolytic metabolism has been linked to the development of chemoresistance in acute myeloid leukemia (AML). However, the underlying mechanisms remain unclear, as well as how to take advantage of this knowledge to improve response to chemotherapy. Our aim was to clarify the role of glucose metabolism in the acquired resistance of AML cells to cytarabine (Ara-C) and to assess its potential as a therapeutic target.

To induce resistance, AML cells were exposed to a stepwise increase in Ara-C concentrations. The resulting Ara-C-resistant cells were characterized for growth rates and doubling time using the Trypan blue assay. Mutation analysis was performed by next generation sequencing (NGS)-based using the Archer Variant Plex® Core Myeloid gene panel, and the metabolic profile was characterized by the Seahorse technology (OCR, ECAR, GlycoPER). Response to the different metabolic inhibitors was evaluated by Trypan blue.

Ara-C resistant KG-1 cells (KG-1 Ara-R) displayed growth rates similar to parental cells, while MOLM13 Ara-R grew at a slower rate than parental cells. Induction of Ara-C resistance triggered loss of NRAS mutation in KG-1 cells, and acquisition of a novel CEPBA mutation in MOLM13 cells. Metabolically, KG-1 Ara-R displayed higher oxygen consumption (OCR) and glycolytic proton efflux (GlycoPER) than KG-1 cells, while no significant differences were observed for the pair MOLM13/MOLM13 Ara-R cells. KG-1 Ara-R cells exhibited a decreased acute response to 3-bromopyruvate (3-BP) initially but increased sensitivity after 48 hours, and a greater susceptibility to phenformin, compared to parental cells. Conversely, induction of Ara-C resistance did not sensitize MOLM13 cells to metabolic inhibitors.

These findings suggest that acquired resistance to Ara-C in AML may involve metabolic adaptations, presenting opportunities for therapeutic intervention in patients who developed resistance to classical chemotherapy.

60. Exploring the Regulatory Functions of 5-Methylcytosine in Prostate Cancer

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Abstract

Introduction: Prostate cancer (PCa) ranks as the 2nd most incident cancer and 5th most leading cause of cancer-related deaths worldwide. Its heterogeneous nature poses a significant challenge in patient treatment and management. Thus, there is an urgent need for the discovery of new molecular signatures that improve patient stratification and refine therapeutic approaches.

5-Methylcytosine (m5C) is one of the most prevalent RNA modifications in mammals. Its regulatory proteins, writers, readers and erasers, have been linked to various cancers, including PCa. Furthermore, m5C modification has been identified as a potential predictive biomarker guiding treatment decisions in other malignancies.

However, our comprehension of the role of m5C modification in PCa remains limited. Thus, the main aim of this study is to thoroughly investigate and characterize m5C modification and their associated regulatory proteins in PCa.

Materials and Methods: An in-silico analysis was conducted using The Cancer Genome Atlas (TCGA) to assess m5C related proteins in PCa. The transcript levels of the most dysregulated genes, namely, NSUN2, NOP2, NSUN6 and YTHDF2 were evaluated in a cohort of PCa patients who underwent prostatectomy at IPO Porto. NSUN2 and NOP2 protein levels were also assessed in castration-resistant C4-2 and C4-2B PCa cell lines, derived from LNCaP cell line. NSUN2 was silenced by shRNA in C4-2B cells and effects in vitro are being assessed along with RNA sequencing.

Results: Several m5C players, including NSUN2, were significantly deregulated in TCGA PCa cohort. m5C players levels did not associate with aggressiveness in PCa patients' samples. PCa cell lines displayed contrasting NSUN2 and NOP2 protein levels, with higher expression observed C4-2B than in C4-2. Successful downregulation of NSUN2 was attained in C4-2B cells. The associated functional assays and RNA sequencing are still in progress.

Conclusions: Our preliminary results support a significant involvement of m5C regulatory proteins in PCa progression.

61. Beyond Li-Fraumeni: Challenges and Implications of TP53 Variant Analysis in Hereditary Cancer Risk Assessment

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Abstract

Introduction: Germline TP53 variants are traditionally linked to Li-Fraumeni syndrome (LFS), typically diagnosed through family history and specific cancers. Comprehensive cancer panels have identified TP53 variant carriers not meeting classic criteria, suggesting a broader spectrum of cancer risk.

Despite the availability of established guidelines for TP53 variant classification, certain variants remain challenging. One example is the c.845G>A p.(Arg282Gln) variant.

The objective of this study was to analyse this variant by examine its frequency within our cohort, reviewing family phenotypes and available classification data.

Patients and methods: Retrospective review of all patients who underwent TP53 gene testing and positive for the c.845G>A variant. Data collected included the following clinical characteristics: gender, personal/family history of cancer and tumor characteristics. Also, we conducted a review of available data regarding the c.845G>A variant.

Results: From 4352 index patients tested for TP53 variants, 7 (0.16%) harbored the c.845G>A variant. Preliminary analysis of clinical data reveals variability in phenotypes compared to classical TP53 phenotypes. Although data review found conflicting results in functional assays, the mutant protein still loses transcriptional activity on key target genes in both yeast and lymphoblastoid cell lines.

Conclusion: Identification of pathogenic/likely pathogenic germline TP53 variants allows recognition of LFS patients and significantly impacts decisions regarding surveillance strategies, risk reduction measures and family planning. TP53 surveillance guidelines has created challenges, such as pressure on the national healthcare system and concerns about the psychological burden of frequent WB MRIs. These factors highlight the critical need for a more comprehensive risk stratification approach and a better understanding of modifier factors, particularly when dealing with possible low penetrance TP53 variants.

62. Exploring Tumor-Hematopoietic Interactions in Colorectal Cancer Immune Evasion

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Abstract

Introduction: The immunoediting comprises an initial elimination phase targeting nascent tumor cells, followed by an equilibrium phase wherein the immune response and tumor cells enter a state of homeostasis, temporarily constraining tumor expansion. Tumors eventually escape immunosurveillance, leading to the development of detectable benign tumors and escalating the risk of malignant progression. Our aim was to identify the genetic, cellular and molecular drivers of escape to immunosurveillance.

Materials and methods: We used genetically engineered Msh2/Kras mouse models to study colorectal carcinogenesis, gathering samples from the spleen, bone marrow, thymus, small intestine, and corresponding tumors at approximately 20 and 40 weeks of age. Tissue sections were paraffin-embedded for hematoxylin and eosin staining to complement our analysis. To prepare for flow cytometry analysis, we processed a portion of the tissues into a single-cell suspension and assessed several markers. Additionally, tissue sections were paraffin-embedded for hematoxylin and eosin staining to complement our analysis.

Results: Oncogenic Kras accelerated tumor growth in a Msh2 null background compared with single mutants. The Msh2/Kras mice displayed multiple tumors throughout the intestine, splenomegaly, signs of anemia, alongside histologic and cytologic abnormalities within the bone marrow and the thymus. A detailed flow cytometry analysis of the spleens of Msh2/Kras tumor-bearing mice revealed alterations in distinct immune cell populations compared with age-matched wild-type and tumor-free single mutant mice. Additionally, the number of tumors positively correlated with spleen size and a negatively correlated with the percentage of CD45+ cells in the spleen. The putative mediators of the crosstalk between tumors and the hematopoietic organs were evaluated through multiplex cytokine array. Several cytokines exhibited differential alterations, shedding light on their role in the crosstalk. We are now addressing how these alterations impact the tumor immune microenvironment.

Conclusions: Our study highlights a novel immunosurveillance escape mechanism involved in oncogenic KRAS-driven intestinal tumorigenesis through long-distance communication with hematopoietic organs. This knowledge is crucial for the development of novel therapeutic strategies aimed at bolstering the immune response against nascent tumors, impeding their escape, and ultimately curbing the progression rate toward malignancy.

63. Deciphering the role of cholesterol in the tumor microenvironment: novel colorectal cell line clustering approach

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Abstract

Introduction: Cholesterol, whose metabolism tends to be dysregulated in colorectal cancer (CRC), has the potential to promote an immunosuppressive tumor microenvironment (TME). An in silico study conducted within our research group found that CRC can be clustered into groups based on the differential expression of several genes involved in cholesterol metabolism. The results also show differences between the clusters regarding the TME, implying that cholesterol may indeed modulate the immune response in the context of CRC. Our aim was to replicate these findings with CRC cell lines, creating a system for studying the immunomodulatory potential of these clusters in vitro.

Materials and Methods: RNAseq raw counts from 55 cell lines from the Cancer Cell Line Encyclopedia were downloaded, normalized through EDA Seq normalization, and converted to log2. Single-sample Gene Set Enrichment Analysis (ssGSEA) was employed to score them according to the expression levels of genes within six curated gene sets (cholesterol biosynthesis, efflux, storage, reverse transport, uptake, and catabolism). The k-means algorithm was used to divide the samples into five distinct clusters. We then explored whether these clusters presented differences regarding genes that could intervene in the modulation of the immune response in the TME, using ssGSEA to compare the clusters regarding several pathways related to immunomodulation.

Results: We found statistically significant differences between the clusters regarding the expression of genes related to adaptive immune response and, more specifically, antigen processing and presentation, correlating with their cholesterol metabolism.

Conclusions: Our findings suggest a potential link between cholesterol metabolism and immune modulatory properties of cell lines, which can thus serve as a model for studying the effects of cholesterol in the TME. Further validation studies are underway using cell lines representative of each cluster to confirm these results.

64. Genetic alteration in Rab27a enhancer linked to inflammatory signatures and prognosis in pancreatic cancer

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Abstract

We have recently shown that pancreatic ductal adenocarcinoma (PDAC) is associated with a marked decrease in Rab27a gene expression, a gene pivotal for exosomes secretion. Furthermore, we have described an enhancer region within the Rab27a locus, and confirmed its regulatory impact on gene expression.

In here, we have screened for genetic alterations of the Rab27a enhancer, uncovering specific alterations in the PANC-1 human PDAC cell line, which were absent in the normal HPNE ductal cell line. We extended our analysis to human PDAC tissue samples, detecting these genetic changes in 2 out of 10 cases. Notably, the samples with the alteration showed low and no Rab27a expression.

In a larger cohort of human PDAC samples, we discovered that a significant portion, 25%, exhibited no Rab27a expression in their primary tumors. Correlating with these findings, tumors characterized by low Rab27a expression levels were heavily infiltrated by MRP8+ inflammatory myeloid cells and were linked to poorer prognoses than their high-expression counterparts.

Altogether, our study identifies the first genetic alteration within the Rab27a gene in human PDAC, which may play a role in the inflammatory profile and high aggressive potential of these tumors.

65. Patient-derived organoids as ex-vivo experimental models for precision medicine in pediatric brain tumors

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Abstract

Pediatric brain tumors (PBT) are the leading cause of non-accidental death in pediatric age. PBT are rare and lack adequate experimental models capable of accurately capturing their complex molecular landscape. Our team has established specific protocols for the generation and expansion of ex-vivo patient-derived organoids (PDO) from fresh surgical material of a variety of PBT, within a clinically relevant timeframe. These include a variety of low-grade and high grade gliomas, embryonal tumors, an ependymoma and a meningioma. The PDO cultures were set within 7-15 days, with PDO reaching 200-800 µm in diameter. Immunofluorescence analysis using confocal high-content microscopy revealed that PDO kept the protein markers that are typically found in the corresponding primary tumors. Likewise, at the genomic level, PDO recapitulated the molecular landscape of the corresponding primary tumors, as denoted by the maintenance of NF1, PIK3CA, and FGFR1 mutations (rosette-forming glioneuronal tumor), KIAA1549::BRAF fusions (pilocytic astrocytomas), ZFTA::RELA fusion (supratentorial ependymoma), gene amplifications, such as KRAS, CDK4, MDM2, GLI1, PTPN11 and PI3KCA (diffuse high-grade glioma), among others. Additionally, PDO exhibited minimal genetic drift after over one month in culture. In three PBT cases from a medulloblastoma, an atypical teratoid rhabdoid tumor and a meningioma, where no molecular alterations were detected using a comprehensive NGS panel, we performed DNA methylation array and found that PDO methylation profile closely matched those of primary tumors. Altogether, our results underscore the potential of PDO as a robust ex-vivo experimental model. The generated PDO cultures can be rapidly established and retain key molecular features of the corresponding primary tumors. This model has versatile applications, serving as a valuable molecular tool for precision medicine approaches, with a great potential to be used as a preclinical model for finding new therapies in the context of PBT patients.

66. Breaking bad: cancer-associated fibroblasts challenge KRAS-targeted therapy in colorectal cancer

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Abstract

KRAS-targeted therapies have emerged as promising treatments for a subset of colorectal cancer (CRC) patients with KRAS mutations. However, rapid onset of resistance to therapy impairs their efficacy. Here, we aim to explore the role of cancer-associated fibroblasts (CAFs) in fostering therapy resistance in CRC and its possible clinical implications.

CRC cell lines HCT15, HCT116 and SW480 harboring KRAS mutations were cultured either in recommended media or in conditioned media (CM) from normal colon fibroblasts cell line (CCD-18co) either activated with TGF-β (CAFs) or not. Expression of membrane stem cell markers (SCM) was analyzed by flow cytometry (FC). Stem cell potential was evaluated by in vitro sphere forming assay. RNAseq analysis was performed in KRAS silenced HCT116 colonospheres treated with either control media or CM of CAFs.

Basal FC expression in the three CRC cell lines revealed heterogeneous SMC signatures, unique to each cell line.

Upon KRAS inhibition, CRC cells exhibit decreased stemness, marked by increased CD24 expression, reduction of integrins CD49f and CD104 and impaired sphere-forming efficiency (SFE). Remarkably, exposure to CAF-derived factors counteracted the inhibitory effects of KRAS silencing, rescuing the stem-like phenotype and reinstating SFE.

RNAseq analysis demonstrated that CAF-secreted factors stimulate the activation of pro-tumorigenic pathways in KRAS-silenced cells, including cell cycle progression, epithelial-to-mesenchymal transition (EMT), NOTCH signaling, and immune modulation. Furthermore, it increased cellular activity and exit from quiescence.

These findings highlight a novel mechanism that shows that CAF-derived factors can induce resistance to KRAS inhibition by promoting a stem-like phenotype and by enhancing several pro-tumorigenic pathways in a KRAS-independent manner. Targeting CAF-induced stemness and associated signaling pathways could enhance the efficacy of KRAS-targeted therapies overcome its current pitfalls.

67. Dissecting the role of PGRMC1 as a potential CSC biomarker associated with breast cancer brain metastasis

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Abstract

Breast cancer (BC) is a major cause of cancer death, mainly due to distant metastases. In particular, brain metastases are a major concern due to the lack of effective therapies, decreased survival rates, and poor quality of life of the patients. Although cancer stem cells (CSCs) represent a small subpopulation within the tumour, they are described as being involved in metastasis formation. Therefore, the search for new CSC biomarkers and improvements in CSC-targeted therapies are crucial to tackle metastatic progression. In this work, our major goal was to specifically identify membrane CSC biomarkers and dissect their potential role in breast cancer brain metastasis.

For that, the MDA-MB-231 metastatic BC cell model and its organotropic variant for the brain were used. Through membrane proteomic analysis, PGRMC1 was identified as a potential CSC biomarker in brain metastatic breast cancer cells. After PGRMC1 chemical inhibition with AG-205, a significant decrease in mammospheres formation was observed for the brain organotropic variant, confirming an impact on the stem properties of brain tropic BC cells in vitro. Importantly, using a cohort of primary breast tumours, high PGRMC1 expression was associated with clinicopathological features, as well as with a CSC profile. Moreover, a significant association was observed between strong PGRMC1 expression and a worse 5-year overall survival.

In summary, PGRMC1 not only showed to impact on the stem properties of brain tropic BC cells in vitro but was also associated with a CSC profile and worse prognosis for BC patients. Therefore, further work should be performed to consolidate the relevance of PGRMC1 as a biomarker for CSCs associated with breast cancer brain metastasis as well as a prognostic factor and therapeutic target.

68. Enhancing triple-negative breast cancer immunotherapy: chitosan/γ-PGA nanoparticles as adjuvants to IFN-γ

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Abstract

Interferon-γ (IFN-γ) plays a crucial role in antitumor immunity and immunotherapy, yet its efficacy as a standalone therapy is limited. Combining IFN-γ with other immunotherapies, is under clinical investigation. Our previous research introduced chitosan/poly(γ-glutamic acid) nanoparticles (Ch/γ-PGA-NPs) as a biomaterials-based immunotherapy, capable of enhancing the effects of other cancer therapies. These NPs reprogram immature/immunosuppressive antigen-presenting cells (APCs) into immunostimulatory ones, activating T cells and inhibiting APC-mediated cancer cell invasion. Recently, we showed that Ch/γ-PGA-NPs, when used as adjuvants to radiotherapy, reduced breast tumor burden, lung metastasis, and systemic immunosuppression. Therefore, the immunomodulatory properties of Ch/γ-PGA-NPs make them promising candidates as adjuvants to IFN-γ-based therapies.

Herein we investigated the synergistic effects of Ch/γ-PGA-NPs and IFN-γ using a 4T1 breast tumor model. Animals were subcutaneously injected with NPs, IFN-γ, or both for two weeks following 4T1 cells inoculation. Results showed that neither treatment affected mice weight or liver/kidney function, indicating their safety. While untreated animals exhibited progressive tumor growth and lung metastasis, those treated with NPs or IFN-γ alone experienced a significant reduction in tumor burden. Combining both treatments resulted in the complete inhibition of tumor growth. This effect was accompanied by reduced splenomegaly, a decrease in myeloid-derived suppressor cells, and an increase in antitumoral T helper 1 cells. Animals treated with NPs+IFN-γ also showed lower levels of systemic pro-tumoral cytokines and lung metastasis compared to untreated animals. Furthermore, ongoing spatial imaging analysis aims to provide additional insights into the immunophenotype profiling of tumors. Overall, Ch/γ-PGA-NPs synergize with IFN-γ to inhibit tumor progression and systemic immunosuppression, offering new avenues for anticancer strategies.

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