10-11 MAY 2018

3RD ASPIC INTERNATIONAL CONGRESS

PROCEEDINGS BOOK

Fundação Calouste Gulbenkian

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3RD ASPIC International Congress

ASPIC
ASSOCIAÇÃO PORTUGUESA DE INVESTIGAÇÃO EM CANCRO

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LETTER OF WELCOME

We are pleased to welcome you to the 3rd ASPIC International Congress at the Gulbenkian Foundation, in Lisbon, May 10-11, 2018.

Why come to the ASPIC Congress at a time when you receive on a daily basis advertisement for high level meetings all over the world? Meetings where you can get in touch with experts and colleagues and share experiences and develop new ideas for your work! In our view the answer is because you can get that at the ASPIC Congress as well! With several advantages: it is a small and more informal meeting and it has a fantastic program covering hot topics for basic researchers and clinical researchers alike. And what's more, you will liaise with Portuguese cancer researchers working in Portugal and abroad contributing to reinforce cancer research in Portugal.

At a time when the research flows every day information on new achievements and when new (and incredibly expensive) drugs are also coming into the clinic at a high pace, the existence in each and every country of an active research community is the most relevant protective umbrella to avoid shortsightedness in public awareness and in political decisions that affect all of us and our families.

The 3rd ASPIC Congress will give cancer researchers a stage where they speak to the inside and to the outside of the community, to patient's associations and to the public, on their successes but also on the many still unsolved questions. Speak what we know, and what we don't know. At times of explosive information as we are living, the need for critical discussion without preconceptions is more important than ever.

The 3rd ASPIC Congress is coming back to the Gulbenkian Foundation where the first Congress in 2014 gathered for the first time 249 researchers, mostly Portuguese cancer researchers. A number that was scaled up to 425 participants at the 2nd ASPIC Congress at IPO-Porto in 2016. We look forward to have you all in Lisbon sharing ideas to improve the life of cancer patients in the years to come. Gulbenkian adds to the scientific standard of the Congress the beauty of the venue and a fantastic city to visit again and again.

Join us in Lisbon next May!

Jorge Soares
Congress President

Leonor David
Congress Vice-president

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CONGRESS COMMITEES

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President: Jorge Soares

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CONGRESS PROGRAMME

THURSDAY, 10 MAY

09.00 **Opening Session**
Jorge Soares, Leonor David and Luís Costa

Plenary Lectures
Chairs: João Barata, Leonor David

INVITED SPEAKERS

09.30 EACR Lecture – Anton Berns (President of EACR; Netherlands Cancer Institute) | *Mouse models of lung cancer. What do they teach us?*

10.15 ASEICA Lecture – Xosé Bustelo (Vice-President of ASEICA; Universidad de Salamanca) | *Bivalents roles of Vav oncoproteins in cancer: Genetic evidence and potential translation to human settings*

11.00 Coffee break

Symposium I
INVASION AND METASTASIS
Chairs: Joana Paredes and Bruno Costa Silva

SELECTED ORAL PRESENTATIONS

11.30 Paulo de Sepúlveda (CRCM, INSERM) *Unexpected tumor suppression function for FES tyrosine kinase in cancer*

11.45 Carlos Custódia (IMM) | *Patient-derived xenograft models of brain metastases for translational medicine*

INVITED SPEAKERS

12.00 Susana Godinho (Barts Cancer Institute) *Oxidative stress in cells with extra centrosomes drives non-cell autonomous invasion*

12.30 Sandra Swain (Georgetown University Medical Center) | *The path to success in HER2-positive breast cancer*

13.00 Lunch

14.00 **Poster Session - Rooms 1, 2 and Hall**

Symposium II
THERAPY RESISTANCE
Chairs: João Nuno Moreira and Isabel Fernandes

INVITED SPEAKERS

16.00 Peter Nelson (Fred Hutchinson Cancer Research Center) | *Therapy Resistance: The Emergence of New Cancer Species Evolving Through Treatment Pressures*

16.30 Daniel Peeper (Netherlands Cancer Institute) | *Towards rational combinatorial cancer treatment — a functional genomics approach*

SELECTED ORAL PRESENTATIONS

17.00 Vânia Palma Roberto (UAlg) | *TERT methylation: a potential diagnostic and prognostic tool for the management of colorectal cancer*

17.15 Raquel Cruz-Duarte (IMM) | *PLCγ1 Mediates Resistance to Anti-EGFR Therapy in Metastatic Colorectal Cancer*

17.30 Coffee break

18.00 **ASPIC General Assembly**

20.00 Congress Dinner

FRIDAY, 11 MAY

Parallel workshops

09.00

WORKSHOP 1
The importance of biomarker validation to increase success in cancer drug development and approval. *(Organization: Júlio Oliveira, Lúcio Lara Santos, André Albergaria) - Room 3*

WORKSHOP 2
Consortium Kick-off Meeting: Building and promoting Excellence in Cholangiocarcinoma Sciences in Portugal. | *(Organization: Júlio Oliveira, Lúcio Lara Santos, André Albergaria) - Auditorium 3*

WORKSHOP 3
Presentation of the European Reference Network GENTURIS - Genetic tumour risk syndromes: opportunities to improve health care for patients with hereditary cancer syndromes in Europe. *(Organization: Carla Oliveira and Tamara Hussong Milagre (patient association EVITA) - Auditorium 2*

SPEAKER
Nicoline Hoogerbrugge (GENTURIS President)

ASPIC Plenary Lecture

Chairs: Luís Costa and Fátima Vaz

10.15 Carlos Caldas (University of Cambridge)
A systems level panoramic view of breast cancer

11.00 Coffee break

Symposium III
NEW TECHNOLOGIES / NEW ACHIEVEMENTS

Chairs: Raquel Seruca and Lúcio Lara Santos

INVITED SPEAKERS

11.30 Leon Alkalai (NASA) | *The Future of Robotic Space Exploration: from our Solar System to Interstellar Exploration. How Medicine can benefit from that knowledge?*

12.00 Luís Almeida (Blueclinical, Universidade do Porto) | *Oncological R&D Pipeline – booming of mechanisms and targets*

12.30 J. Iñaki Martin-Subero (IDIBAPS)
Decoding the epigenome of normal and neoplastic B cells: biological and clinical insights

13.00 Lunch

Symposium IV
METABOLISM AND CANCER

Chairs: Ana Preto and Manuel Sobrinho Simões

INVITED SPEAKERS

14.00 Michael P. Lisanti (University of Salford)
Cancer Stem Cells (CSCs): New Approaches to their Identification and Eradication

14.30 Carla Martins (University of Cambridge)
Targeting the metabolic dependencies of mutant Kras lung tumours

SELECTED ORAL PRESENTATIONS

15.00 Soraia Melo (i3S/Ipatimup) | *A novel regulation mechanism underlying loss of E-cadherin expression in Hereditary Diffuse Gastric Cancer families*

15.15 Bruno A. Cardoso (IMM) | *CASZ1 is overexpressed in T-cell acute lymphoblastic leukemia and promotes cell transformation and tumor growth via PI3K-mTOR*

15.30 Coffee break

Symposium V
HOST AND CANCER (IMMUNOTHERAPY)

Chairs: Sofia Braga and José Carlos Machado

INVITED SPEAKER

16.00 Christian Blank (Netherlands Cancer Institute) | *Neo-adjuvant checkpoint inhibition – the pathway towards personalized immunotherapy*

SELECTED ORAL PRESENTATIONS

16.30 Celine Gonçalves (ICVS, UMinho)
Functional and prognostic relevance of WNT6 in human glioblastoma

16.45 Maria José Oliveira (i3S/INEB)
Reprogramming antigen-presenting cells: a chitosan/γ-PGA nanoparticles for anticancer therapy

INVITED SPEAKER

17.00 Cora Sternberg (San Camillo Forlanini Hospital) | *Immunotherapy in the treatment of Urothelial Cancer (UC)*

17.30 **EACR awards and closing**
Carlos Caldas, José Luís Passos Coelho, Jorge Soares and Luís Costa

ABSTRACTS

PLENARY LECTURES
Thursday 10 and Friday 11 May

EACR LECTURE

PL 1. *Mouse models of lung cancer. What do they teach us?*

Anton Berns

Lung cancers belong to the most lethal human malignancies. In particular, patients with small cell lung cancer (SCLC), and lung squamous cell carcinoma (LSCC) show very poor survival statistics due to the late detection, early metastatic spread, and chemo-resistance of the tumors. We have generated multiple mouse models for lung cancer subtypes and studied how closely they resemble their human counterpart, how these tumors develop over time, from which cells they originate, what additional genomic alterations are recurrently found and how this influence their response to single drugs or drug combinations. We show that the mouse tumors show remarkable similarity with the cognate human tumors. This includes their marker profile, their location within the tissue, their immunophenotype and their refractoriness to treatment. We observe substantial intratumor heterogeneity and tumor plasticity, this in spite of the fact that the mouse tumors do not exhibit a high mutation load. Also the cell-of-origin of these tumors can be quite diverse. The lessons learned from these models will be discussed.

A.B. studied biochemistry at the University of Nijmegen and received his Masters degree in 1969 and his PhD in 1972 from that same University, both with honors. He did his postdoctoral training in the group of Rudolf Jaenisch at the Salk Institute in La Jolla, CA., where he studied the role of retroviruses in causing lymphomas in mice. In 1976 he returned to the University of Nijmegen where he became junior staff member. His group explored proviral insertional mutagenesis as a means to identify new oncogenes. In 1985 he was appointed as staff scientist at the Netherlands Cancer Institute and in 1986 he became head of the Division of Molecular Genetics of the Institute. In 1999 he was appointed as Director of Research and Chairman of the Board of Directors of The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital. He retired from that position at the end of 2011. He is member of several academies: The Netherlands and European Academy of Sciences, The European Academy of Cancer Science, he is EMBO member, fellow of the AACR and foreign member of the American Academy of Sciences. He also has received a number of prestigious prizes for his research. A.B. research lab is interested in defining the genetic aberrations that are critical for lung tumor development.

ASEICA LECTURE

PL 2. *Bivalents roles of Vav oncoproteins in cancer: Genetic evidence and potential translation to human settings.*

Xosé Bustelo

Rho GDP/GTP exchange factors (GEFs) are enzymes that promote the activation of Rho GTPases in both normal and cancer cells. Due to this, it is widely assumed that they can represent potential anticancer drug targets. However, we still have very little information about the actual role of these enzymes in human cancer, the therapeutic effectiveness of inhibiting their enzyme activities, and the side effects that such inhibition elicits in healthy tissues. Our group is trying to address these issues using as main tool the three known members of the Vav GEF family, the proteins Vav1, Vav2 and Vav3. To this end, we are utilizing a multifaceted approach based on the use of genetically modified mice, genome wide expression profiling techniques, cross-species

transcriptomal comparisons, and patient-derived cell models. In my talk, I will present recent data demonstrating that the endogenous wild type and mutant versions of these proteins play proactive roles in mature T cell leukemia and a variety of epithelial tumors. Furthermore, I will discuss data from our "pharmaco-mimetic" knock-in mice demonstrating, for the first time in the field, that the inhibition of GEFs does have a negative impact on the fitness of tumor cells in vivo. Perhaps more importantly, these studies also have revealed the feasibility of dissociating the positive and negative effects elicited by the inhibition of Vav proteins depending on the level of catalytic silencing achieved at the organismal level. On the other side of the coin, I will present new results showing that a member of this family, Vav1, can unexpectedly play catalysis-independent tumor suppression roles in immature, TLX+ T-cell acute lymphoblastic leukemia. The elimination of this function seems to be critical for the pathogenesis of this leukemia subtype. These observations underscore the complex functional landscape regulated by these proteins as well as the pros and cons associated with the potential use of anti-Vav therapies in the near future.

This work is supported by grants from the Spanish Ministry of Economy and Competitiveness (SAF2015-64556-R, CB16/12/00351), the Castilla-León Government (CSI049U16), Worldwide Cancer Research (14-1248), Ramón Areces Foundation, and the Spanish Association against Cancer (GC16173472GARC).

X.B. is a Full Research Professor at the Spanish National Research Council (CSIC) and a principal investigator at the Cancer Biomedical Research Center (CIBERONC). X.B. scientific training includes a Ph.D. in Biology from the University of Santiago de Compostela (Galicia, Spain, 1990) and a postdoctoral stint at the Bristol–Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, USA; years 1990–1993). He then worked as Staff Scientist at the Bristol–Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, USA; years 1993-1996), as Assistant Professor at the Department of Pathology of the State University of New York at Stony Brook (Stony Brook, NY, USA; years 1996–2000) and, subsequently, as both CSIC Tenured (1999–2004) and Senior Research (2004–2005) scientist at the Cancer Research Center (Centro de Investigación del Cáncer, CIC) of Salamanca (Spain). X.B.'s work has been focused on the understanding of signal transduction processes initiated by Rho GTPases during both physiological and pathological conditions. His group has made key findings on the regulation of these proteins, the isolation of upstream GTPase regulators, and the elucidation of the role of these proteins in normal physiological contexts and high–incidence pathologies such as cancer, cardiovascular disease, and metabolic syndrome. X.B. scientific output includes 123 scientific articles and 9 patents. His work has been recognized by a number of both Spanish and international scientific awards. X.B. is the Vice-Director of the CIC of Salamanca, the Director of the CIC Genomics and Proteomics Unit, a member of the CIBERONC Executive Committee, the coordinator of the CIBERONC Mechanisms of Tumor Progression Program, and the President–Elect of ASEICA.

ASPIC LECTURE

PL 3. ***A systems level panoramic view of breast cancer.***

Carlos Caldas

(no abstract available)

C.C. is Professor of Cancer Medicine, University of Cambridge, and Head of Breast Cancer Functional Genomics Laboratory, Cancer Research UK Cambridge Institute. C.C. is an Honorary Consultant Medical Oncologist and Breast Cancer Programme Director at the Cambridge Cancer Centre. He is Fellow of the Academy of the Medical Sciences, Fellow of the European Academy of Cancer Sciences, and EMBO Member. He received the 2016 ESMO Hamilton Fairley Award and holds an ERC Advanced Grant (2016-2021). His research focus is the functional genomics of breast cancer and its biological and clinical implications. His laboratory redefined the molecular taxonomy of breast cancer, demonstrated the role for miRNAs as modulators of the immune response, explored the clonal heterogeneity of triple negative breast cancers and the patterns of whole-genome ER binding in primary tumors, established ctDNA as a monitoring biomarker in breast cancer and pioneered the use of patient-derived tumor explants as a model for breast cancer. He has published over 300 manuscripts, including in Nature, Cell, NEJM, Nature Genetics, Science Translational Medicine and Nature Communications.

ABSTRACTS

Symposium I
INVASION AND METASTASIS
Thursday 10 May

INVITED SPEAKER

IS1. Oxidative stress in cells with extra centrosomes drives non-cell autonomous invasion.

Susana Godinho

The centrosome is the main microtubule-organising centre in animal cells; important to assemble a bipolar mitotic spindle ensuring proper chromosome segregation and genomic stability. Centrosomal abnormalities, in particular centrosome amplification, are recurrent features of human tumours. Enforced centrosome amplification in vivo plays a role in tumour initiation and progression. However, centrosome amplification occurs only in a subset of cancer cells and thus, partly due to this heterogeneity, the contribution of centrosome amplification to tumours in unknown. Here, we show that supernumerary centrosomes induce a paracrine-signalling axis via the secretion of proteins, including interleukin 8 (IL8), which leads to non-cell autonomous invasion in 3D mammary organoids and zebrafish models. This extra centrosomes-associated secretory phenotype (ECASP) promotes invasion of human mammary cells via HER2 signalling activation. Further, we demonstrate that centrosome amplification induces an early oxidative stress response via increased NOX-generated reactive oxygen species (ROS), which in turn mediates secretion of pro-invasive factors. The discovery that cells with extra centrosomes can manipulate the surrounding cells highlights unforeseen and far-reaching consequences of these abnormalities in cancer.

S.G. did her undergraduate degree in Biology – Microbiology and Genetics at the University of Lisbon. After that, she did her PhD at Institute Gulbenkian of Science (Portugal) and Cambridge University (UK) where she studied the role of Polo kinase during mitosis. Following her PhD, Dr. Godinho moved to Boston where she did her postdoctoral studies at Dana-Farber Cancer Institute and Harvard Medical School. It was during this time that she became interested in understanding how and why cancer cells accumulate extra numbers of centrosomes. Her work had important implication for the development of selective therapeutic strategies that target cancer cells and had a key role in the understanding how centrosome amplification could contribute to tumourigenesis. In August 2013, Dr. Godinho moved to London to set-up her own lab at Barts Cancer Institute, QMUL. Her main research interest is to investigate how cancer cells adapt to and maintain high levels of extra centrosomes and to determine how centrosome amplification impacts on cell physiology to promote tumourigenesis.

INVITED SPEAKER

IS2. Treatment of HER2 positive advanced Breast Cancer.

Sandra M. Swain

Anti-HER2 targeted therapy has changed the trajectory of lives of patients with HER2 positive breast cancer. The results in the adjuvant and metastatic trials show a large survival benefit with the use of these therapies. In the past, a patient with HER2 positive breast cancer would have the worse prognosis, now it is among the best. Most recently the CLEOPATRA trial utilizing pertuzumab with trastuzumab in advanced disease first line treatment reported a 15.6-month improvement in median survival which was an unprecedented finding. There continues to be development of other HER2 targeted therapies since patients with metastatic disease are not cured. There is a great interest in combining these agents with CDK4/6 inhibitors in patients with ER positive,

HER2 positive disease. Since there is a known immunomodulatory effect of trastuzumab it is also speculated that immunotherapy in combination will be effective.

S.S., MD, FACP, FASCO is a Professor of Medicine and the Associate Dean for Research Development at the Georgetown University Medical Center (GUMC) in Washington, DC. She is also an Adjunct Professor of Medicine at the Uniformed Services University of the Health Sciences. S.S. graduated with a Bachelor of Arts (B.A.) in Chemistry from the University of North Carolina in 1975 and earned her Doctor of Medicine (M.D.) from the University of Florida in Gainesville in 1980. She completed a residency in Internal Medicine at Vanderbilt University in 1983 followed by a fellowship in Medical Oncology at the National Institutes of Health, National Cancer Institute in 1986. She served at the National Institutes of Health as the Deputy Branch Chief for the Medicine Branch of the Center for Cancer Research at the NCI as a tenured Principal Investigator. Next at the NIH, she became the Head of the Breast Cancer Section, and Chief of the Cancer Therapeutics Branch. From 2007-2016 she served as the Medical Director of the Washington Cancer Institute at MedStar Washington Hospital Center in Washington, DC. Swain's research interests include translational research and clinical trials focused on metastatic and inflammatory breast cancer, adjuvant therapy for breast cancer, cardiotoxicity, and health care disparities. She has published over 270 articles and has received numerous awards and recognitions for her work, including the Susan G. Komen Award of Distinction for Community Service, the NIH Merit Award, NCI Mentor of Merit Award, the Claude Jacquillat Award for Clinical Cancer Research and the 3rd Aleksandr Savchuk Foundation Prize. S.S. served as President of the American Society of Clinical Oncology (ASCO) from 2013-14 and sits on the Executive Committee of the Lombardi Comprehensive Cancer Center at GUMC. She is an active member of the NRG Breast Committee and Fellow of the American College of Physicians and ASCO.

C5. Unexpected tumor suppression function for FES tyrosine kinase in cancer.

Tisserand, J, Desaunay, M and De Sepulveda, P

Cancer Research Center of Marseille, CRCM, INSERM, CNRS, Institut Paoli-Calmettes and Aix-Marseille University, France

Introduction: FES is one of the pioneer tyrosine kinases identified about 35 years ago. FES was initially discovered as the product of the feline v-fes and avian v-fps viral oncogenes. c-FES has since then been considered as a proto-oncogene. However, no convincing evidence for the implication of FES in human pathology had been provided so far. Also, despite many descriptive studies, the molecular and physiological functions of FES remain obscure.

Materials and Methods: We have used original and modified cell lines, mouse models of melanoma and panels of human melanoma tumors and genomic data to characterize the role of FES in carcinogenesis. The analyses included large scale mutation, gene expression, DNA methylation analyses and immunohistochemistry. Furthermore, large scale analysis of mouse tumors and mass spectrometry were used to investigate the molecular partners and pathways related to FES function.

Results and discussion: We have investigated c-FES mutations in human tumors. Surprisingly, we did not find any gain-of-function mutation; instead, we identified several loss-of-function mutations. Since FES mutations are rare, we then looked for loss of expression as another mean to generate FES loss-of-function in cancer cells. The pattern of expression of c-Fes is restricted to a limited number of cells in normal physiology. The analysis of cultured cells revealed unexpected expression of c-FES in normal melanocytes and loss of expression in the vast majority of melanoma cell lines and primary cultures. Loss of expression is due to massive DNA CpG methylation in c-FES promoter region and can be reverted upon treatment with DNMT inhibitors. Importantly, we provide compelling evidence for a tumor suppression function for FES in melanoma using a combination of in vitro cell-based assays, mouse models of melanoma and, analysis of human melanoma tumors (results published in JCI 2017). In addition, our analyses of gene expression and mass spectrometry provide hypotheses for the molecular mechanism involved in FES function. These include well-known pathways as well as the description of the main FES protein partner in cells. In conclusion, we demonstrate for the first time the implication of FES in human cancer; we reveal that FES has tumor suppression activity and that its expression can be reverted using demethylation agents. Furthermore, our work brings to light novel perspectives for FES molecular function in normal and pathological context.

No conflict of interest.

E1. Patient-derived xenograft models of brain metastases for translational medicine

Cláudia C. Faria [1,2], Carlos Custódia [1], Eunice Paisana [1], Rita Cascão [1], João T. Barata [1]

[1]Instituto de Medicina Molecular - João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;
[2]Serviço de Neurocirurgia, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisboa, EPE, Lisboa, Portugal

Introduction: Brain metastases (BMs) affect approximately 40% of patients with any given cancer. Despite the current available treatments, including surgery and radiation therapy, BMs are incurable, and patients have a dismal outcome. The molecular heterogeneity of BMs suggests the need for personalized therapeutic approaches. Therefore, the development of appropriate animal models of human BMs is crucial to validate novel treatment strategies.
Materials and Methods: Fifteen BMs with different primary origins were collected during surgery in the Department of Neurosurgery at Hospital de Santa Maria (Lisbon). Immunocompromised mice were used to develop patient-derived xenograft (PDX) models of BMs. Human samples were implanted subcutaneously in the flank of NSG mice, and serially passaged. Engrafted tumors were harvested, dissociated into a single cell suspension and injected in the heart of NSG mice. Animals were euthanized upon reaching the humane endpoint and the central nervous system (CNS) along with other organs were collected for histological analysis, to assess the pattern of cancer cell dissemination. Patient-derived cultures were also established and used for drug testing. Response to drugs was assessed through proliferation assays and western blot.
Results and discussion: The overall take rate in the subcutaneous model was 66% and it correlated with patient survival (P=0.0032). In many flank implanted BMs, there was spontaneous dissemination of cancer cells to multiple organs, including the CNS, mimicking patients' disease. Of notice, samples from lung BMs developed spontaneous metastases in the lung, suggesting that these tumor cells maintained the ability to return to the primary organ.
The intracardiac injection of tumor-isolated cells increased their metastatic potential, particularly to the CNS. Interestingly, in five PDXs from diverse primary tumors, the location of BMs in the mouse brain recapitulated the location in the patients' brain.
Finally, we assessed the efficacy of targeted antineoplastic agents, currently in use in the clinic, in the established patient-derived cultures of BMs. Buparlisib (pan-PI3K inhibitor) and everolimus (mTOR inhibitor) successfully reduced cancer cell proliferation and induced specific pathway inhibition.
We conclude from our work that patient-derived models of human BMs recapitulate patient's disease and can be a powerful tool in the development of novel targeted therapies to treat metastatic cancer.
No conflict of interest

ABSTRACTS

Symposium II
THERAPY RESISTANCE
Thursday 10 May

INVITED SPEAKER

IS3. Therapy Resistance: The Emergence of New Cancer Species Evolving Through Treatment Pressures

Peter Nelson

Metastatic cancers are generally treated with agents designed to damage DNA or impair cell division. More recently, these malignancies are treated with small molecules and antibodies designed to selectively inhibit specific signaling pathways or augment anti-tumor immunity. Though initial responses are often substantial, most advanced cancers develop resistance and progress. Notably, recent studies have demonstrated the remarkable plasticity of tumor cells to assume new cell identities capable of bypassing inhibition of the treatment target. This talk will focus on advanced prostate cancer and the evolution of novel cellular phenotypes that develop as a consequence of cancer treatment. Importantly, these new tumor cell identities are accompanied by new treatment targets that may be predictable and emphasize the importance of combination therapy.

Peter Nelson is a Member in the Divisions of Human Biology and Clinical Research at the Fred Hutchinson Cancer Research Center and Professor of Medicine at the University of Washington in Seattle, Washington. He is a medical oncologist with a clinical practice focused on developing and evaluating novel therapeutics. His research centers on identifying molecular alterations in prostate cancer that contribute to metastasis and therapy-resistance. His research group identified major components of the gene expression program regulated by the androgen receptor (AR) and identified molecular mechanisms that drive resistance to AR-targeted therapeutics. In collaborative studies through the International SU2C/PCF Prostate Cancer Dream Team, his group identified inherited determinants of aggressive prostate cancer and defined the landscapes of molecular aberrations in advanced prostate cancer that classify subtypes for precision oncology.

INVITED SPEAKER

IS4. Towards rational combinatorial cancer treatment — a functional genomics approach

Daniel S. Peeper

For a long time, advanced-stage melanomas were refractory to the available therapeutic options, but recent developments have begun offering better perspectives for patients. The small molecule inhibitor vemurafenib, specifically targeting the mutant BRAFV600E kinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually most melanomas become resistant and progress on treatment. This occurs by the acquisition of additional mutations or other alterations, most of which reactivate the mitogen-activated protein kinase (MAPK) pathway. Although further suppression of BRAF-MAPK signaling by the inclusion of MEK inhibitor delays resistance, eventually most patients relapse.

The clinical outcome of late-stage melanoma patients has also greatly improved thanks to the recent availability of T cell checkpoint modulation, primarily by CTLA-4 and PD-1/PD-L1 blockade. But still, large patient groups fail to (durably) benefit from these treatments, underscoring the continuing need for developing novel therapeutic modalities.

Therefore, in spite of these new perspectives, there is a dire need to identify additional targets amenable to therapeutic intervention, possibly to be used in combination settings with tumor inhibitors alongside immune activators. We are studying (lack of) sensitivity to both tumor and immune cell treatment using patient biopsies, patient-derived xenografts

(PDX) and low-passage cell lines. These systems are used for systematic function-based genetic screens to identify melanoma and immune cell factors representing pharmacologically tractable therapeutic targets. The results from these and related studies will be discussed.

Daniel S. Peeper, PhD Head, Division of Molecular Oncology & Immunology, Netherlands Cancer Institute Permanent Staff member, Group leader Chair of the Research Faculty Council, Netherlands Cancer Institute Chair of the Translational Research Board, Netherlands Cancer Institute Professor of Functional Oncogenomics, VU University Medical Center, Amsterdam Member of Oncode Institute. D.P. is professor in Functional Oncogenomics heading the Department of Molecular Oncology & Immunology and chairing both the Research Faculty Council and the Translational Research Board at the Netherlands Cancer Institute (NKI). He has received several awards, including a KWF Queen Wilhelmina Award and a Society for Melanoma Research (SMR) Outstanding Researcher Award. He is an elected Member of Oncode (a funded network of selected cancer scientistsin the Netherlands), EMBO and Academia Europaea and serves on several Boards, including that of the European Association for Cancer Research. His laboratory discovered the physiologic relevance and mechanism of Oncogene-Induced cellular Senescence as a tumor-suppressing mechanism in humans. More recently, his team has been focusing on translational cancer research, using function-based genetic screens for drug target identification and unraveling mechanisms mediating resistance, both for tumor and immune cell therapies. Several potential new tumor and immune cell drug targets have been identified and are being studied in preclinical models. Most recently, his group dissected the mechanism of cancer drug addiction and provided PoC of how this vulnerability may be used clinically, and developed a new rational concept for combinatorial targeting of intratumor heterogeneity.

B7. TERT methylation: a potential diagnostic and prognostic tool for the management of colorectal cancer

Vânia Palma Roberto, Joana Apolónio and Pedro Castelo-Branco

Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal; Centre for Biomedical Research, University of Algarve, Faro, Portugal; Algarve Biomedical Center, University of Algarve, Faro, Portugal

Introduction: Colorectal cancer (CRC) is a leading cause of death by cancer worldwide. Despite the increasing research, relapse still occurs in about 50% of the patients. Most cancers are able to recur by gaining limitless self-renewal. This hallmark of cancer is governed by telomere maintenance, achieved by telomerase activation in 90% of human tumors. Telomerase Reverse Transcriptase (TERT) reactivation in cancer is regulated by both genetic and epigenetic events. In the last years we have shown that hypermethylation of a specific region of the TERT promoter named TERT Hypermethylated Oncological Region (THOR) was associated with TERT expression and patient outcome in other cancers. Here, we aimed to uncover the potential of TERT methylation as a biomarker for CRC and explore the underlying mechanisms.
Materials and Methods: Gene expression, DNA methylation and clinical data for CRC patients were retrieved from The Cancer Genome Atlas (TCGA) Colorectal Cancer Cohort (n=736). RNA was extracted from CRC cell lines depleted or silenced for DNA methyltransferases (DNMTs). DNMTs and TERT expression was assessed by qPCR. For groups comparisons Mann-Whitney test or one-way ANOVA, followed by Kruskal-Wallis and Dunn's Multiple Comparison tests, were used. For clinical significance, we considered the area under the ROC curve (AUC), sensitivity and specificity and patient overall survival (OS), determined by Kaplan-Meier curves and log-rank test.
Results and Discussion: Our analysis revealed that TERT expression and THOR methylation were significantly increased in CRC malignant tissue ($p<0.0001$). THOR could distinguish early stages of cancer from normal tissue (AUC=0.8627; 81.27% sensitivity and 75.55% specificity, $p<0.0001$) evidencing its potential as a biomarker candidate for CRC. We then started to explore the mechanisms behind TERT methylation by unravelling the DNMT responsible for this epigenetic event. In HCT116 cells, TERT expression was downregulated upon DNMT1 depletion indicating that this enzyme plays a central role on TERT methylation. Our data further suggested that methylation does impact on TERT expression and to explore its clinical significance we analysed the methylation of the entire TERT gene. We found 13 CpG sites that were differentially methylated between normal and tumor tissues and correlated with THOR. In fact, we discovered a potential combinatory biomarker able to predict patient outcome. Lowly methylated patients for THOR, cg14647640 and cg25353384 had a

chance of survival around 90% versus 20% survival (p=0.0008) of the highly methylated group. Moreover, this TERT methylation pattern might help discriminate high-risk from low-risk patients within CRC stages II and III.
In conclusion, we showed that THOR has independent diagnostic value in CRC and that its combination with other methylation events of the TERT gene could predict CRC patients' outcome.
Acknowledgements: VPR and JA were the recipients of the LPCC-Fundação PT2016 and PD/BD/105899/2014 FCT fellowships, respectively. This work was co-financed by UID/BIM/04773/2013 CBMR and Maratona da Saúde.
No conflict of interest

I5. PLCγ1 Mediates Resistance to Anti-EGFR Therapy in Metastatic Colorectal Cancer

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Introduction: Tumor metastases are responsible for approximately 90% of all cancer-related deaths. Cetuximab (Cetx) is a clinically approved EGFR-targeted monoclonal antibody used in the treatment of metastatic colorectal cancer (mCRC). However, Cetx effectiveness is low due to the existence of multiple resistance mechanisms that converge in the constitutive activation of downstream pathways of EGFR. Namely, RAS mutations are known to induce resistance to upstream blockage. Nevertheless, still 40% of RAS wild-type patients do not respond to these treatments. Therefore, there is a clear need for new biomarkers capable of accurately predict response to therapy. Phospholipase C γ1 (PLCγ1) is an effector of EGFR, involved in oncogenic signaling downstream of this receptor. In this context, we found that PLCγ1 is involved in Cetx resistance in an innate and acquired manner.
Materials and Methods: We have analyzed, by Immunohistochemistry, levels of PLCγ1 expression in human CRC samples, and correlate it with responses of patients to Cetx. To support this data, in vitro PLCγ1 knockdown and overexpression in CRC cell lines allowed us to identify PLCγ1 contribution to Cetx resistance. We also evaluated PLCγ1 levels in CRC cells that acquired resistance to Cetx by continuous exposure to this drug. To validate our in vitro experiments, we xenotransplanted PLCγ1 knockdown and control cells in Zebrafish embryos and evaluate tumor growth in the presence of Cetx.
Results and Discussion: Our analysis of PLCγ1 expression in human CRC samples showed a significant correlation between increased PLCγ1 expression and poor progression-free survival in mCRC patients under Cetx treatment, suggesting an involvement of PLCγ1 in the resistance to Cetx. Furthermore, in a panel of five CRC cell lines, levels of PLCγ1 expression were higher in cells showing increased resistance to Cetx. Knocking down PLCγ1 in these cell lines sensitizes them to Cetx treatment, while overexpression of PLCγ1 increases their resistance. Additionally, cells exposed to Cetx for 5 months showed increased levels of PLCγ1 when compared to parental cells, suggesting an effect of this protein also in an acquired resistance to Cetx. Zebrafish PLCγ1 knockdown xenografts treated with Cetx have reduced tumor burden and increased caspase 3 activity when compared with control xenografts. These results indicate that PLCγ1 is possibly involved in evading apoptosis under to Cetx treatment. We are currently investigating the mechanism how PLCγ1 allow cells to escape Cetx-induced cell death. Overall, our results show that PLCγ1 is involved in an intrinsic and acquired resistance to Cetx, allowing cells to overcome apoptosis induced by cetx. Ultimately, these results indicate that PLCγ1 could be a predictive biomarker of responses to Cetx treatment and a putative therapeutic target.
No conflict of interest

ABSTRACTS

Symposium III
NEW TECHNOLOGIES / NEW ACHIEVEMENTS
Friday 11 May

INVITED SPEAKER

IS5. The Future of Robotic Space Exploration: from our Solar System to Interstellar Exploration. How Medicine can benefit from that knowledge?

Leon Alkalai

(no abstract available)

Leon Alkalai is the Assistant Division Manager for Formulation and the Lead for Formulation at Jet Propulsion Laboratory, California Institute of Technology, Engineering and Science Directorate. Leon is a recently appointed JPL Fellow and he is a Full Member of the International Academy of Astronautics (IAA). Leon received his PhD in Computer Science from UCLA in 1989. For the first 15 years at JPL L.A. was a leader in Advanced Avionics Systems, Micro-Systems, Micro/Nano Spacecraft and related technologies. For the past 10 years, Leon has been in the forefront of JPL's competed missions' project formulation as a Manager and a Business Capture Lead. Leon was the successful Capture Lead for both the GRAIL mission to the Moon: awarded in 2007 and launched in 2011; and then the InSight mission to Mars: awarded in 2012 and to be launched in 2016. Both competitions were part of NASA's Discovery Program in Solar System Exploration. In 2012, Leon received the NASA Individual Distinguished Achievement Medal for the successful formulation of the GRAIL mission to the Earth's Moon.

INVITED SPEAKER

IS6. Oncological R&D Pipeline – booming of mechanisms and targets

Luís Almeida

The pharmaceutical R&D pipeline is facing a remarkably growth and the field of oncology is the most active. In this presentation, the statistics of active R&D projects are reviewed by type of sponsor, type of product, phase of development, geographical region and therapeutic category, disease/indication, delivery route, mechanism of action and targets.

Luis Almeida is Founder and Managing Director at Blueclinical Ltd, a CRO especially devoted to translational medicine and early clinical development. With a M.D. and a Ph.D. in Medicine, he is a specialist in Clinical Pharmacology and Pharmaceutical Medicine and has over 30 years of experience in drug development. Luis Almeida is President of the College of Clinical Pharmacologists of the Portuguese Medical Association and Affiliate Professor at the Faculty of Medicine, University of Porto. Main research activities/interests include translational medicine, drug development and clinical trials. He is inventor/co-inventor of 3 international patents.

INVITED SPEAKER

IS7. Decoding the epigenome of normal and neoplastic B cells: biological and clinical insight.

J. Iñaki Martin-Subero

(no abstract available)

J.I.M.-S. graduated from the University of Navarra (Spain) with a degree in Biochemistry. In 2001, he completed a PhD with honours as a joined effort between the University of Navarra and the Christian-Albrechts University of Kiel (Germany). He continued his postdoctoral training at the Christian-Albrechts University and in 2005 he became faculty member. Upon returning to Spain in 2009, he started to coordinate a research group on epigenomics at the University of Barcelona. In 2016, he was appointed leader of the Biomedical Epigenomics group at the IDIBAPS research institute in Barcelona, where he currently leads a group of 8 researchers. He has published over 140 peer-reviewed articles with over 7000 citations in Scopus and an H index of 47. Dr. Martin-Subero's research is focused on the application of advanced technologies to characterize epigenomic marks in normal and neoplastic lymphoid cells. His current efforts are focused on understanding gene deregulation in lymphoid tumours through the integration of DNA methylation, histone modifications, chromatin accessibility and 3D chromatin structure. His ultimate goal is that the generated epigenomic knowledge can be translated into a benefit for patients, in terms of better diagnosis, estimation of prognosis and more appropriate treatments.

ABSTRACTS

Symposium IV
METABOLISM AND CANCER
Friday 11 May

INVITED SPEAKER

IS8. Cancer Stem Cells (CSCs): New Approaches to their Identification and Eradication.

Michael P. Lisanti

In my talk, I will discuss the use of cell metabolism to define the cancer stem cell (CSC) phenotype and how metabolic flexibility and synthetic lethality can be combined to eradicate CSCs with FDA-approved drugs and dietary supplements, such as Doxycycline and Vitamin C.

I began my education at New York University, graduating in Chemistry. I obtained my MD-PhD degrees at Cornell University Medical College in Cell Biology and Genetics. From 1992-96, I was a Fellow at the Whitehead Institute at MIT. After several distinguished appointments at the Albert Einstein College of Medicine and the Kimmel Centre, I joined the Breakthrough Breast Cancer Research Unit in 2012 as Professor of Cancer Biology, at The University of Manchester, in the United Kingdom (UK). Following my appointment to the Kimmel Cancer Centre in 2006, I was selected for the leadership of the Program in Molecular Biology and Genetics of Cancer. In 2009, I became the Chair of the Department of Stem Cell Biology and Regenerative Medicine at Thomas Jefferson University. I also served as the former Editor-in-Chief of the American Journal of Pathology. In Manchester, I held the Muriel Edith Rickman Chair of Breast Oncology. I have lectured in various MD and PhD level graduate courses in Biochemistry, Cell Biology, Pharmacology, Pathology and Clinical Medicine, among others. Research Interests: Anti-Cancer Therapies and Anti-Ageing Therapies.

INVITED SPEAKER

IS9. Targeting the metabolic dependencies of mutant Kras lug tumours.

Carla Martins

Lung cancer is currently the most lethal cancer worldwide and lung adenocarcinoma its most common histological subtype. The vast majority of lung cancer patients present with locally advanced, inoperable or metastatic disease and median survival at this stage remains low. Targeted therapies are already improving treatment outcomes, but frequent mutations such as those affecting KRAS or TP53 (p53) (present in ~30% and ~50% of lung adenocarcinomas, respectively) remain untargetable. Our lab aims to identify new vulnerabilities associated with these aggressive, heterogeneous and largely therapy-resistant tumours. To achieve this, we are characterizing the mechanisms that drive the malignant progression of mutant Kras, p53-deficient lung tumours, using mouse models that closely recapitulate human lung adenocarcinoma. Using a multidisciplinary approach, we recently identified distinct genetic, transcriptional and metabolic signatures between low grade and high-grade mutant Kras lung tumours. In particular, we showed that high grade mutant Kras lung adenocarcinomas frequently exhibit extra copies of mutant Kras and undergo metabolic rewiring. We demonstrated that the gain of a single mutant Kras gene copy (single vs double mutant) has a major effect on lung tumour cells in vitro and in vivo, leading to enhanced metastatic potential and glucose metabolism reprogramming. In turn, this metabolic rewiring creates unique dependencies that can be exploited to selectively target advanced mutant Kras lung tumours. Taken together, our data indicate that mutant Kras lung tumours are not a single disease but are instead comprised of two classes of tumours that exhibit mutant Kras gene dosage-dependent prognosis and therapeutic susceptibilities. This intrinsic tumour heterogeneity is also

present in the human disease and may have contributed to the poor therapeutic responses often associated with mutant KRAS lung tumours in humans. We have also shown that distinct p53 mutations can differentially impact lung tumour metabolism and are therefore also likely to contribute to the metabolic heterogeneity of lung tumours. We will discuss these findings and how unique metabolic signatures can potentially be exploited to improve lung cancer targeting in the clinic.

C.M. is a MRC Programme Leader at the MRC Cancer Unit, University of Cambridge, UK. Carla received her MSc in Biology and Genetics from Moscow State University, Faculty of Biology, Russia. In 1996 she joined the Gulbenkian PhD Programme in Biology and Medicine (Portugal), and subsequently carried out her PhD work at The Netherlands Cancer Institute in the lab of Prof. Anton Berns (1997-2003). Carla then moved to UCSF, USA for her Postdoctoral training in the lab of Prof Gerard Evan (2003-2009) and later joined the lab of Prof David Tuveson at the CRUK Cambridge Institute, UK as an Associate Scientist (2009-2011). In 2011 Carla became a group leader at the MRC Cancer Unit, where she heads the Modelling Tumour Development and Therapy group. The aim of her lab is to define the molecular and functional alterations that enable lung tumour progression from benign stages to advanced disease using a combination of in silico, in vitro and in vivo approaches. The current focus of her work is on the identification and pre-clinical targeting of the genetic, transcriptional and metabolic alterations responsible for the progression of mutant Kras lung tumors, with the ultimate aim of developing improved cancer diagnostic and therapeutic tools for this disease.

A7. A novel regulation mechanism underlying loss of E-cadherin expression in Hereditary Diffuse Gastric Cancer families

Joana Figueiredo [1,2#], Soraia Melo [1,2,3#], Kimberley Gamet [4], Tanis Godwin [5], Susana Seixas [1,2], João M. Sanches [6], Parry Guilford [5], Raquel Seruca [1,2,3*]
# These authors have equal contribution to the work

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Introduction: Germline loss-of-function mutations in E-cadherin gene (CDH1) are a proven cause of a cancer syndrome called Hereditary Diffuse Gastric Cancer (HDGC), and appear in approximately 40% of all HDGC families [1-3]. HDGC is characterized by the occurrence of diffuse-type gastric carcinoma and lobular breast cancer (LBC) and to date, more than 155 CDH1 germline mutations were described in this syndrome [3-5]. Clinicians and many researchers have disregarded mutations occurring in E-cadherin signal sequence since this portion is cleaved upon translocation to the endoplasmic reticulum (ER) and is not part of the mature protein [6-8]. Stressing this notion, we have found a novel HDGC family that presents a strong aggregation of gastric cancer and carries a mutation in the signal peptide of E-cadherin (c.38_46del). Given that the hydrophobic core of signal peptides serves as docking site for the signal recognition particle (SRP), the main responsible for detecting the translocation code of secretory and membrane proteins [9 10], we hypothesized that disruption of this portion might disturb protein processing and explain the E-cadherin-mediated gastric cancer cases exhibited by this family. Taking advantage of our well established approach that combines expression profiling, functional assays and bioimaging techniques [3 5 11 12], we demonstrated that the variant is pathogenic, affecting the stability, localization and the adhesive/anti-invasive function of E-cadherin with consequences on epithelia structure and organization. Moreover, this mutation do not change E-cadherin mRNA levels but delays its translation and translocation to the ER, hindering its expression rescue to the plasma membrane by the treatment with chemical chaperones or inhibition of the proteasome. Overall, we have unveiled the mechanisms underlying the deleterious effects of germline mutation c.38_46del and exposed a novel post-

translational regulatory process associated to HDGC families, which support the significance of E-cadherin signal region in this setting. Accordingly, the identification of germline mutations within this domain should be from now on regarded as causative genetic event of HDGC and considered in other cancer syndromes.

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No conflict of interest

A.23 CASZ1 is overexpressed in T-cell acute lymphoblastic leukemia and promotes cell transformation and tumor growth via PI3K-mTOR

Cardoso, B.A.[1*], Gírio, A.[1*], Fragoso, R.[1*], Martins, L.R.[1], Moreira, A.S.[1], Correia, N.C.[1], Bortolini, A.[2], Grosso, A.R.[1], Almeida, S.F.[1], Yunes, J.[2], Pflumio, F.[3], Barata, J.T.[1]
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Introduction: Introduction. CASZ1 is a zinc finger transcription factor essential for blood vessel assembly, heart morphogenesis and neural development. CASZ1 acts as tumor suppressor in neuroblastoma but was recently shown to promote metastasis in ovarian cancer. Here, we sought to characterize the involvement of CASZ1 in T-cell acute lymphoblastic leukemia (T-ALL).
Material and Methods. CASZ1 expression in T-ALL patients was determined from publicly available data and real-time PCR. CASZ1 and TAL1 were modulated in T-ALL cell lines, patient samples and IL3-dependent BaF3 cells by retroviral transduction. Impact on proliferation and cell viability was determined by flow cytometry. The CASZ1 transcriptional program was determined by RNA-sequencing. BaF3-empty and BaF3-CASZ1 expressing cells were subcutaneously transplanted into NOD/SCID mice and tumor volume was measured to assess tumorigenic impact. The clinical-grade PI3K-Akt-mTOR pathway inhibitor Dactolisib was used to inhibit the pathway in vitro and in vivo.
Results and Discussion. We show that T-ALL patient samples, particularly TAL1-positive cases, overexpress CASZ1. In agreement, TAL1 forced expression in T-ALL cells upregulates CASZ1, whereas TAL1 silencing reduces CASZ1 expression. Furthermore, ChIP-PCR showed that TAL1 binds to the CASZ1 locus in T-ALL cells, suggesting that TAL1-dependent transcription is one of the mechanisms by which CASZ1 is upregulated in T-ALL. To unravel the functional impact of CASZ1, we silenced CASZ1 and found that partial knockdown reduces T-ALL cell viability and proliferation. In contrast, stable overexpression of CASZ1 rescues the viability and expansion of BaF3 cells cultured in the absence of IL-3. Interestingly, KEGG and GO term analyses of differentially

expressed genes in BaF3-CASZ1 cells versus BaF3-empty controls reveal enrichment for cancer-associated pathways, including PI3K-Akt-mTOR. In agreement, this pathway is constitutively active in BaF3-CASZ1 cells and its integrity is mandatory for CASZ1-dependent proliferation and viability. Moreover, BaF3-CASZ1 cells originate large tumor masses in vivo whereas none of the controls develop tumors. Notably, tumor growth is significantly delayed using Dactolisib, further highlighting that CASZ1 mediates leukemogenesis, at least in part, by activating PI3K signaling. In summary, our studies demonstrate that CASZ1 is overexpressed and acts as an oncogene in T-ALL, identifying a previously unrecognized regulator of T-ALL biology.

No conflict of interest

ABSTRACTS

Symposium V
HOST AND CANCER (IMMUNOTHERAPY)
Friday 11 May

INVITED SPEAKER

IS10. Neo-adjuvant checkpoint inhibition – the pathway towards personalized immunotherapy.

Christian Blank

Targeted and immunotherapy have revolutionized the systemic and adjuvant therapy in late stage melanoma. Recent pre-clinical and clinical data indicate that neoadjuvant immunotherapy might be superior to adjuvant therapy. Neoadjuvant therapy can bear several advantages, allowing one to 1) determine therapy efficacy within the individual patient for possible additional adjuvant therapy, if needed, 2) reduce tumor burden before surgery, 3) utilize pathological response data as surrogate outcome markers for relapse free and overall survival, and 4) identify biomarkers in a homogenous patient population. For these reasons (except latter), neoadjuvant therapy has become a standard of care in high tumor burden breast cancer. In the case of T cell checkpoint blockade, neoadjuvant therapies could bear a fifth, and potentially significant, advantage. T cell checkpoint blocking antibodies enhance T-cell activation at the moment antigen is encountered, and drug exposure during the time the in-situ malignancy still provides a major source of antigen may therefore potentially induce a stronger and broader tumor-specific T cell response. I will present pre-clinical and clinical data supporting this notion and will give an outline for effective biomarker identification, that will lead towards personalized combination immunotherapy in melanoma and beyond.

C.B. obtained his MD from the Medical School of the Technical University Munich, Germany, where he also completed his Doctoral thesis at the Department for Medical Microbiology and in 1997. As a Junior House Officer (1997–1998) Dr Blank held four positions at the Departments of Cardiology and of Radiology at the University Clinic Munich Rechts der Isar, Munich, the Accident and Emergency (A&E) Departments at the Royal Infirmary of Edinburgh, Scotland, and the University of Birmingham, England, UK. He went on to attain the position of Physician at the Department of Haematology and Oncology, University of Regensburg, Germany (1998–2001). During 2001–2003, Dr Blank held a Postdoctoral Research Fellowship at the lab of Professor Thomas Gajewski, University of Chicago, IL, USA. Subsequently he was appointed as Physician and Research Group Leader at the Department of Haematology and Oncology, University of Regensburg, Germany (2003–2007). Dr Blank has obtained two Specialist Degrees in Internal Medicine (2007) and in Haematology/Oncology (2009). Since 2007, he has been appointed Staff Member at the Department of Medical Oncology, and Group Leader at the Division of Immunology, The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital (NKI-AVL), Amsterdam. In 2010, Dr Blank became University Lecturer (Privatdozent) at the University of Regensburg. Obtained his Master of Business Administration (MBA), from the University of Warwick, England, UK (2006). Appointed Professor in 2015 in Medical School of the University of Regensburg. C.B.is author of more than 90 publications and his research interests include targeted and biological response modifiers in melanoma, and prognostic markers in melanoma.

INVITED SPEAKER

IS11. Immunotherapy in the treatment of Urothelial Cancer (UC).

Cora N. Sternberg

(no abstract available)

C.N.S. is chief of the Department of Medical Oncology at the San Camillo-Forlanini Hospital in Rome, Italy. She has attained the rank of full Professor of Oncology and is an adjunct professor at La Sapienza University in Rome. She is also adjunct professor of Urology and Urological Oncology at Tufts University School of Medicine and the Lahey Clinic in Boston, Massachusetts, and adjunct professor at Temple University's College of Science and Technology in Philadelphia, Pennsylvania. A graduate of the University of Pennsylvania undergraduate and medical schools, she completed her fellowship and was on staff at Memorial Sloan Kettering Cancer in NY. C.N.S. has published more than 370 articles, 6 textbooks, and is an associate editor and editorial board member of several International journals. European School of Oncology (ESO) core faculty and in tune with American medicine, Dr. Sternberg has been elected for a 3-year term to the nominating committee of ASCO's board of directors.

A18.Functional and prognostic relevance of WNT6 in human glioblastoma

Gonçalves, CS [1,2], Castro, JV [1,2], Pojo, M [1,2], Martins, EP [1,2], Taipa, R [3], Pinto AA [4], Faria CC [5,6], Reis RM [1,2,7], Sousa N [1,2], Costa BM [1,2]

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Introduction: Glioblastoma (GBM) is the most common and malignant type of glioma, for which novel therapies targeting specific underlying oncogenic events are urgently needed. While the Wnt pathway has been shown to be frequently activated in GBM, constituting a potential therapeutic target, the relevance of WNT6, an activator of this pathway, remains unknown. Materials and Methods: WNT6 expression was evaluated in GBM at the mRNA and protein level and was silenced or overexpressed in GBM cells to assess functional effects in vitro and in vivo. WNT6-signaling pathways were identified using phospho-kinase arrays, and significant associations with stem-cell features and cancer-related pathways were validated in patients. Survival analyses were performed with Cox regression and Log-rank tests. Results and Discussion: We show that WNT6 is significantly overexpressed in GBMs, as compared to lower-grade gliomas and normal brain, at mRNA and protein levels. In vitro WNT6 expression was associated with increased viability, glioma stem-cell capacity, invasion, migration, proliferation, and resistance to temozolomide chemotherapy. Mechanistically, we identified typical oncogenic pathways, including Src and STAT, which intertwined with the WNT pathway may be critical effectors of WNT6-associated aggressiveness in GBM. In in vivo orthotopic GBM mice models, WNT6 associates with shorter overall survival and increased features of tumor aggressiveness, in both overexpressing and silencing models. Concordantly, in several independent cohorts, WNT6 expression was associated with GBM patients' shorter survival. This study unravels novel functional, mechanistic and clinical implications of WNT6 in glioblastoma, implicating it as an important oncogenic factor and a potential biomarker in this tumor. These findings may help on patient stratification and design novel attractive therapeutic options for this deadly disease.

No conflict of interest

J2. Reprogramming antigen-presenting cells: a chitosan/γ-PGA nanoparticles for anticancer therapy

Flávia Castro [1,3], Marta L. Pinto [1,3], Andreia M. Silva [1,3], Graciosa Q. Teixeira [1,3], Catarina L. Pereira [1,2], José H. Teixeira [1,3], Maria G. Lázaro [1,2], Susana G. Santos [1,2], Mário A. Barbosa [1,3], Olivier de Wever [4,5], Karine Serre [6], Raquel M. Goncalves [1,3*], Maria J. Oliveira [1,2*]

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Introduction: The tumor microenvironment is a complex and dynamic niche that has a fundamental role in tumor cell proliferation, angiogenesis, invasion and metastasis. Macrophages (Mac) are described as having a central role in tumor progression and dendritic cells (DC) as having an immature/immunosuppressive profile, which limits T cells activity. Therapeutic strategies targeting these cells have been emerging as adjuvants to anticancer conventional treatments, aiming at their recruitment and repolarization towards an immunostimulatory phenotype favoring cancer cell elimination. Here, we focused on the potential of Chitosan/Poly(γ-glutamic acid) nanoparticles (NPs) to modulate cellular immunity and, consequently, to affect cancer-cell related activities.

Material and Methods: NPs were prepared by co-acervation method. Primary human monocyte-derived M2 Mac (obtained by stimulation with IL-10) were incubated with NPs. Control experiments with unstimulated Mac and LPS-stimulated Mac (M1 phenotype) were performed. After 72h, cell metabolic activity, cell phenotype and cytokine production were evaluated. Mac ability to induce T cell proliferation/activation and tumor cell invasion were also assessed. The same studies were performed with human-monocyte derived DC. For in vivo models, 1x106 E0771 cells were injected in the mammary fat pad of C57BL/6 mice. After 10 days of tumor implantation, animals were treated every two days with NPs. Tumor growth was assessed by caliper every two days.

Results and Discussion: NPs re-educated IL-10-stimulated Mac towards a pro-inflammatory profile, decreasing CD163 expression and promoting IL-12p40 and TNF-α secretion. NPs also induced an immunostimulatory phenotype on DC, enhancing the expression of the co-stimulatory molecules CD86, CD40 and HLA-DR, and secretion of the pro-inflammatory cytokines TNF-α, IL-12 and IL-6. Interestingly, these phenotypic alterations induced both CD4+ and CD8+ T cell activation/proliferation and counteracted cancer cell invasion, in vitro. Regarding the in vivo tumor model, we observed a decrease in tumor growth in NPs-treated animals comparing to the control, after 23 days of E0771 cell implantation. Additional experiments with other tumor models are currently being explored.

Overall, our findings open new perspectives on the use of NPs as an immunomodulatory therapy for tumor microenvironment reprogramming, providing a new tool for anticancer therapies.

No conflict of interest

POSTER SESSION

Topic A
CELL AND TUMOUR BIOLOGY

A1. The CXCR4/CXCL12 axis in feline mammary carcinoma - a suitable spontaneous model for human breast cancer

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Introduction: The chemokine CXCL12 and its receptor CXCR4 are involved in signaling pathways that control cell survival, migration and proliferation. Evidence for a regulatory role of CXCR4/CXCL12 axis in the progression of the metastatic disease was found in breast cancer patients with the organs and tissues with highest CXCL12 expression frequently showing metastases. On the other hand, CXCR4 is mainly expressed in primary breast cancer lesions and lymph node metastases. In feline mammary carcinoma (FMC), considered as a suitable breast cancer model, the importance of the CXCR4/CXCL12 axis is poorly understood. Taking into account the relevant oncogenic role of the CXCR4/CXCL12 axis in breast cancer progression and its potential to be targeted by anti-tumor molecules, this study aimed to clarify the contribution of the axis in the progression of FMC and metastatic disease. Materials and Methods: The expression of CXCR4 and CXCL12 was analyzed by immunohistochemistry and immunofluorescence on primary tumors (PT), regional (RM) and distant metastases (DM) in cats with mammary carcinoma. Associations between CXCR4 and CXCL12 tissue status and serum CXCL12 values, clinicopathological features and FMC molecular subtypes were also evaluated. Results and Discussion: CXCR4 was more expressed in PT (82.3%) than in RM (70.8%) and DM (54.8%, p=0.0067), whereas CXCL12 was highly expressed in metastatic lesions located in liver and lung (100%), when compared with PT (78.1%, p<0.0001) and as reported for human breast cancer. Moreover, cats with CXCR4-positive PT exhibited significantly lower serum CXCL12 levels (5.16±1.26ng/ml) than cats with CXCR4-negative mammary carcinomas (11.06±3.72ng/ml, p=0.0324). At DM, HER2-overexpressing tumors presented higher CXCR4 expression than other molecular tumor subtypes (100%, p=0.012) revealing a HER2-dependent CXCR4 upregulation. Significant differences in overall (p=0.0147) and disease free survival (p=0.0279) curves between the cats with CXCL12-positive and CXCL12-negative tumors were also found. Indeed, in cats with HER2-overexpressing tumors, CXCL12-negative PT were associated with unfavorable prognosis. In summary, this study exposes the signature of CXCR4 and its ligand CXCL12 in PT but also in metastases of FMC, highlighting the axis as an important target for future therapy and emphasizing FMC as a suitable spontaneous human breast cancer model which may allow predicting novel therapeutic strategies in cats and in humans.

No conflict of interest

A2. Identification of a novel KRAS/Galectin-3/p16INK4a triple axis in colorectal cancer: therapeutic implications

Pereira F. [1,2,3], Cazzanelli G. [2], Carvalho PD. [1], Alves S. [1], Costa AM. [1,3], Pinto ML [1,3], Gomez-Lazaro M. [1,3], Oliveira MJ. [1,3], Preto A. [1,2]

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer-related death. KRAS, Galectin-3 (Gal-3) and p16INK4a have been associated with colorectal carcinogenesis. KRAS is a oncoprotein frequently activated by point mutations contributing to CRC cell survival by inducing autophagy1. P16INK4a is a tumor suppressor protein which loss of function is relevant in colorectal malignant transformation and has been associated with KRAS mutations2. Gal-3 is a multifunctional protein also important in CRC progression that has been correlated with altered cell-cell adhesion, cell-matrix interactions, macrophage activation, cancer cell invasion and tumor angiogenesis3. Although, Galectin-3 and KRAS relationship have been already explored, the triple KRAS/Galectin-3/p16INK4a axis regulation has not been investigated. Thus, we aimed to better understand the interplay of KRAS, Gal-3 and p16INK4a and uncover the role of the complex in CRC. For that purpose, we used normal colon cells transfected with KRAS hotspot mutations (KRASG13D, KRASG12D and KRASG12V) and CRC derived cells.
Co-immunoprecipitation and co-localization results demonstrated for the first time that KRAS/Gal-3/p16INK4a physically interact between them and form a multiprotein complex which showed a feedback loop regulation. We further explored the role of KRAS hotspot mutations in the interplay of KRAS, Gal-3 and p16INK4a and our results indicated that KRAS mutations might be important for the complex regulation. We also explored the impact of KRAS/Gal-3/p16INK4a axis on the survival and invasion of CRC cells. We further showed that KRAS and Gal-3 are important in CRC cell survival decreasing cell proliferation and increasing cell death. In addition, the disruption of the complex significantly decreases CRC cells migration and invasion. Summing-up, this work opens a new field of research, as understanding the role of KRAS/Galectin-3/p16INK4a complex regulation on colorectal carcinogenesis might bring relevant therapeutic implications. [1]Alves,S. et al.2015 "Colorectal cancer-related mutant KRAS alleles function as positive regulators of autophagy." 2Romagosa,C. et al.2011 "p16Ink4a Overexpression in Cancer: A Tumor Suppressor Gene Associated with Senescence and High-Grade Tumors" 3Barrow,H. et al.2011 "The role of galectins in colorectal cancer progression."

No conflict of interest

A3. Deciphering the specifically enriched signature associated with breast cancer brain metastasis

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Introduction: Brain metastases are associated with an alarming poor prognosis, with a mean of 6 months survival after diagnosis, being the metastatic site with the highest impact on the reduced survival of breast cancer patients. Therefore, strategies to predict and prevent this stage of disease are urgent. Poor prognostic basal-like breast cancer (BLBC) metastasizes more frequently to the brain, whereas the good prognostic luminal tumors metastasize mostly to the bone. This data suggests that cancer cells have specific signatures that award them intrinsic advantages to survive in specific organs. Nonetheless, little is known regarding the supporting tissue that provides the appropriate homing conditions, namely the biomechanical properties and the composition of the extracellular matrix (ECM). Actually, within the most common metastatic sites, brain has the most particular physical properties: lowest stiffness and distinct ECM composition, being the organ where the biomechanical crosstalk between cancer cells and ECM is less understood. Thus our goal is to disclose the specifically enriched signature associated with breast cancer brain metastasis and elucidate the paracrine communication between cancer cells and brain pre-metastatic niche. Differentially gene expression profile of MDA-MB-231 BC and brain, bone and lung metastatic variants (231.BR, 231-Bo and 231-Lu) were crossed with different gene ontology signatures. We found that all breast cancer Metastatic cells have an enriched in a matrisome profile. Interestingly, specificity in matrisome classes is observed within metastatic variants: 231.LU show increased CORE-matrisome genes and 231.BR are enriched in ECM-associated regulators genes, which are crucial for ECM remodelling. Additionally, 231.BR cells have a stem-cell/basal-like profile suggesting a link between the basal-like phenotype and the brain metastatic ability. Finally, in vitro zymography confirmed increased protease activity in the secretome from both basal-like and brain tropic BC cells. In conclusion, our

data shows a specific breast cancer brain metastatic signature that most probably will impact brain tissue remodeling in the early steps of the metastatic cascade, preparing the niche for future brain metastasis.

No conflict of interest

A4. ExoCanCell: Targeting Exosomes in Pancreatic Cancer

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with late diagnosis and lack of efficient therapeutic options. PDAC lesions are unique among solid tumors due to their extensive desmoplastic reaction and sparse cancer cells, highlighting the potential role of cell communication in the initiation and progression of this neoplasia. Cell communication, despite mediating different stages of tumor progression, is still off the cancer therapy landscape. Exosomes, extracellular vesicles derived from the endocytic pathway, have emerged as crucial mediators of intercellular communication. The role of cancer exosomes in tumor progression has been widely described. In all stages of exosomes biogenesis Rab GTPases are involved. In here we describe the role of proteins involved in exosomes biogenesis during PDAC progression and therapy response. We show that during PDAC progression Rab-5, -7, -27a and -27b are differently expressed. Increased expression of Rab-27a and -27b correlate with an increase in exosomes number and these features are associated with a more aggressive phenotype. In human PDAC samples, high Rab27a protein levels correlate with poor prognosis. Additionally, cancer cells modulate the biogenesis of exosomes upon gemcitabine treatment, increasing exosomes release. To address our goal, we have generated an inducible Rab27a knockout mouse model conditional to the pancreas, that spontaneously develops PDAC. This model allows us to study the role of exosomes and its biogenesis in disease progression and therapy response, evaluating exosomes-mediated communication as a new therapeutic option in PDAC.

No conflict of interest

A5. AGR2 is a progression marker and a potential therapeutic target in Non-muscle invasive bladder cancer

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Introduction: Bladder Cancer (BC) is the ninth most common cancer worldwide. Non-Muscle Invasive Bladder Cancer (NMIBC) is a heterogeneous disease that includes tumors with low and high risk of progression to muscle invasion. Nowadays, clinico-pathological features alone are not sufficient to effectively classify these patients and the discovery of new biomarkers is essential to increase the quality of their life, who should only be treated with a radical high-morbidity approach, whenever needed, depending on the type of NMIBC tumor developed. ΔNp63 has previously been reported by our group as a protective factor of High Grade (HG) NMIBC progression: From the patients whose tumors showed nuclear ΔNp63 expression, none suffered disease progression after a median follow-up of 62,1 months. Furthermore, when ΔNp63 was knocked down (ΔNKO), cells showed higher proliferation and invasive capacities in vitro and higher tumor and metastasis formation in vivo. Interestingly, ΔNKO cells displayed AGR2 gene up-regulation and protein overexpression. Materials and Methods. Two commercially available HG-NMIBC cell lines (RT112 and BF-TC905) were used to study the role of AGR2 in tumor progression. A stable knockdown of AGR2 (using shRNA technology) was performed on ΔNKO

cells. In vitro functional studies, such as cell cycle, proliferation and invasion assays were performed comparing parental cells, ΔNKO cells and cells with knockdown of both ΔNp63 and AGR2 (AKO). The orthotopic mouse model is being used to study in vivo tumor initiation and metastatic capacities. Immunofluorescence analyses of AGR2 in human HG-NMIBC tissue specimens will be performed to see if AGR2 expression is a prognostic and predictive biomarker for HG-NMIBC. Results and Discussion. After knocking down AGR2 in RT112 and BF-TC905 ΔNKO cells, we observed a reversal of the aggressive phenotype of ΔNKO cells, with lower cell proliferation (cell cycle analysis and proliferation assay) and invasion, thus confirming the functional importance of AGR2. If in vivo results corroborate our hypothesis, we will start a collaboration with Dr. Li's lab to test the efficiency of AGR2 human monoclonal antibody intravesical therapy in NMIBC. This work uncovered AGR2 as a progression biomarker in HG-NMIBC, allowing a better understanding of the mechanisms of BC progression, and opening the possibility of novel therapies for patients affected by this disease, ultimately improving patients overall survival and quality of life.

No conflict of interest

A6. CDH3/P-cadherin expression is associated with aggressiveness of human glioblastoma

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Introduction: Gliomas are central nervous system tumors that include the highly lethal subtype glioblastoma (GBM). Patients diagnosed with GBM exhibit a median overall survival of 15 months. Cadherins are cell adhesion molecules that play an important role in development and, in the last two decades, their involvement in cancer has been established. P(Placental)-cadherin (encoded by CDH3) is known to be relevant in several epithelial tumors. However, the landscape of its functions is still a matter of debate, considering that in some cancers P-cadherin acts as a tumor suppressor gene and in other tumors it has an oncogenic behavior. In this context, we aim to investigate the relevance of P-cadherin in GBM. Materials and Methods: To test the functional importance of P-cadherin in GBM in vitro we used two approaches: CDH3 overexpression in a commercially available GBM cell line (U87MG), and CDH3 silencing in two primary GBM lines established in the group (GL18 and GL42). Functional effects were evaluated by trypan blue and MTS (cell viability), wound healing (migration capacity), Matrigel chamber (invasion), and neurosphere formation assays (stemness features). To evaluate the importance of CDH3 in a more complex system, we established two distinct in vivo models using CDH3-overexpressing cells. We injected U87-Control and U87-CDH3 cells subcutaneously in Nude mice and orthotopically in NOD scid gamma (NSG) mice. We also evaluated CDH3 expression at the protein and mRNA level in glioma samples from our Portuguese cohort. Results and discussion: The in vitro studies revealed that P-cadherin regulates several cancer hallmarks in GBM, such as cell viability, invasion, migration, and stem characteristics. In the in vivo models, U87-CDH3 cells formed larger tumors and caused shorter overall survival of mice. Critically, we found that patients with high grade gliomas present increased number of P-cadherin positive cases at the protein level. Moreover, high CDH3 mRNA levels proved to have a prognostic value in GBM patients, being predictive of poor prognosis. We demonstrated that CDH3/P-cadherin is associated with GBM aggressive phenotype and it is a new prognostic biomarker predictive of shorter survival in patients.

No conflict of interest

A7.

Selected for oral presentation – Symposium IV

A8. The influence of aldh inhibitors in endometrial cancer cells

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Introduction: Endometrial cancer is the most frequent gynecological malignancy in developed countries. Usually, endometrial carcinomas are diagnosed in early stages with good prognosis but treatment for advanced and recurrent disease reveal poor outcomes. Cancer stem cells (CSC) are a minor population that have a determinant role in tumorigenesis, resistance to therapy and metastatic phenotype. Increased expression of detoxifying enzymes, such as aldehyde dehydrogenase (ALDH), in cancer cells is associated with tumor volume, recurrent disease, poor prognosis and contributes to drug resistance. ALDH1 expression in endometrial cancer, was associated with tumorigenesis and chemoresistance. In our previous work, ALDH increased expression was considered a putative marker of endometrial CSC. Material and methods: Two human endometrial cancer cell lines, RL95-2 and ECC-1, were submitted to ALDH inhibitors, diethylaminobenzaldehyde (DEAB) 50-250µM, all-trans retinoic acid (ATRA) 5-10µM and JQ1 100-500nM for 48 hours. Inhibitors cytotoxicity was evaluated through MTT assay. ALDH expression was evaluated through western blot. To perform sphere formation assay, cells were cultured in serum-free DMEM F12 medium with or without 100µM DEAB in appropriate conditions, for 5 days. Spheres were supplemented with EGF and bFGF, every two days, at a concentration of 10 ng/mL. Spheres were photographed and analyzed with Image J software to obtain sphere projection area. Sphere-forming capacity was evaluated. Results and discussion: The presence of ALDH inhibitors does not influence metabolic activity of endometrial cells. ALDH expression of cells submitted to 50 and 100µM DEAB was lower comparing with control cells, about 36% and 27%, respectively. Preliminary results of sphere projection area and sphere-forming capacity showed that spheres submitted to DEAB presented smaller dimensions. Therefore, ALDH inhibition seems to influence the sphere profile. In the future we aim to evaluate the influence of DEAB in the response to chemotherapy. This work was funded by the FCT (PEst-UID/NEU/04539/2013), FEDER-COMPETE (FCOMP-01-0124-FEDER-028417 and POCI-01-0145-FEDER-007440), Bolsa Liga Portuguesa Contra o Cancro/CIMAGO, CIMAGO project nº 02/2017 and Sociedade Portuguesa de Ginecologia/Bayer.

No conflict of interest

A9. The role of CXCR4 in cancer exosomes biodistribution

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Introduction: Exosomes are central mediators of intercellular communication. Exosomes are extracellular vesicles that carry proteins, RNA and DNA of the cells of origin, transfer their cargo to other cells and, at some point, re-educate recipient cells. Cancer exosomes have been involved in almost all steps during tumor progression up to metastasis and therapy resistance. However, the mechanisms underlying the involvement of cancer exosomes in the metastatic process are not fully understood. The CXCR4-CXCL12 chemokine axis is one of the most studied in cancer progression and metastasis. CXCL12 is highly expressed in tissues like lungs, liver,

and bone marrow. CXCR4/CXCL12 interaction results in increased proliferative, migratory, and invasive properties of tumor cells. Although expression patterns vary among cancer types, CXCR4 has been implicated in nearly every major malignancy and plays a prominent role in pancreatic cancer development and progression. Our work is based on the hypothesis that CXCR4+ pancreatic cancer exosomes are preferentially retained at CXCL12-enriched organs. We demonstrate that pancreatic cancer exosomes have a selective loading of CXCR4 receptor at their surface, which could contribute for a differential distribution of cancer exosomes in vivo. Ultimately our work will contribute with new insights into the emerging role of exosomes in cancer and to the understanding of the biological relevance of CXCR4 in pancreatic cancer progression and metastasis.

No conflict of interest

A10. Anti-tumoral effects of diosgenin in prostate cancer cells: insights from cell proliferation, apoptosis, and metabolism

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Introduction: Diosgenin is a phytosteroid from Dioscorea nipponica, which has been reported to have antitumoral activity in several types of cancer. However, a limited number of studies have investigated its effects on prostate cancer (PCa). Also, the mechanisms underlying diosgenin activity remain to be fully elucidated. This work aimed to evaluate the effect of diosgenin in the control of proliferation, apoptosis and glycolytic metabolism of PCa cells. Material and methods: The non-neoplastic cell line PNT1A and the neoplastic prostate cell lines LNCaP, DU145 and PC3 were maintained in culture in RPMI 1640 medium under standard conditions. After 24 h of maintenance in phenol red- and steroid hormone free medium PNT1A, LNCaP, DU145, and PC3 cells were stimulated with diosgenin. Cell viability was assessed using MTT assay, and the IC50 was determined through a range of diosgenin concentrations (0 nM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM and 100 µM). The effect of diosgenin on cell proliferation, apoptosis, and glycolytic metabolism was determined through Western Blot, by analysing the expression of target regulators of these biological processes. The enzymatic activity of caspase-3 and lactate dehydrogenase was determined spectrophotometrically. Moreover, glucose consumption and lactate production were evaluated using commercial kits. Results and Discussion: Diosgenin had an IC50 of, approximately, 40 µM in all the PCa cell lines tested. Besides the observed decrease in PCa cell viability, diosgenin showed to increase caspase-3 activity, indicating augmented apoptosis rates, which was underpinned by the altered expression of proliferation and apoptosis regulators. Diosgenin treatment also changed the glucose consumption and lactate production profile of PCa cells. Altogether, the results obtained, suggest that diosgenin can be a promising agent to be tested in PCa therapy alone or in combination.

No conflict of interest

A11. Clinical Implications of PIK3CA mutations in gliomas molecular subgroups

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Introduction: Gliomas are the most common malignant brain tumors, representing 80% of all malignant neoplasms. Glioblastoma (GBM), the most frequent glioma type has a dismal prognosis. In 2016, the World Health Organization (WHO) classification included for the first time molecular alterations as diagnostic criteria to define gliomas entities. However, still to identify biomarkers that could be used to define better therapies. In this context, PIK3CA mutations have been described as having an important function in several tumors,

showing a putative therapeutic target. In gliomas, it is not clear in which molecular subgroup of gliomas PIK3CA mutations are more frequent and its role as a therapeutic biomarker. In present work we aimed to clarify the clinical importance of PIK3CA mutations in gliomas molecular stratification, prognosis, diagnosis and response to therapy. Material and Methods: Using a cohort of 500 gliomas, classified according with WHO classification 2016, we analyzed PIK3CA mutations on exon 9 and 20 by Sanger sequencing. Until this moment, 218 GBM samples were analyzed (8 GBM IDH-mut and 210 GBM IDH-wt). Results and Discussion: We detected 3.3% (7/210) PIK3CA mutations in GBM IDH-wt, 5 mutations in exon 20 and 2 in exon 9. In the GBM IDH-mut group we found 12.50% (1/8) mutations in exon 20 of PIK3CA. Importantly, we found 2 unreported variants in exon 20 of PIK3CA, c.3112T>C and c.3210A>G. In silico analysis, indicated c.3112T>C as a putative pathogenic variant, while, c.3210A>G as a polymorphic variant. Interestingly 86% of mutations detected in PIK3CA gene had PTEN deletions (6/7), which reinforce the importance PI3K pathway in GBM. Here, we identified, for first time in GBM, one single nucleotide polymorphism (SNP), rs45455192, located in the intronic region flanking exon 9 of PIK3CA. We found 17% and 10% in GBM IDH-wt and GBM IDH-mut, respectively. The presence of this polymorphism did not show statistical differences in overall survival of GBM IDH-wt but these patients showed a short median survival, 11.2 months comparing with 16.4 months in patients without polymorphism. Altogether our results suggest that PIK3CA mutations are predominantly associated with GBM IDH-mut sub-group. Currently, we are analyzing the mutational status of PIK3CA in astrocytomas and oligodendrogliomas molecular subgroups in order to clarify if it is an important player in glioma initiation.

No conflict of interest

A12. Androgen receptor promotes bladder cancer progression

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Introduction: Men are at a higher risk of developing bladder cancer than women. Although the urinary bladder is not regarded as an sex organ, it has the potential to respond to androgen signals. The mechanisms responsible for the gender differences remain unclear. AR after binding with 5α-dihydrotestosteron (DHT) undergoes a conformational change and translocates to nucleus to elicit transcriptional regulation of target genes. However androgen/AR signaling can also be activated by interacting with several signaling molecules and exert its non-genomic function. In response to DHT, androgen receptor can activates PI3K, what results in activation of AKT. Activation of PI3K signaling pathway by AR then may lead to various complex regulatory circuits such as positive and negative feedback loops. We suggest that AR play an essential role in bladder cancer progression in male patients. Studies were carried out on human bladder cancer cell lines: HCV29 (nonmalignant epithelial cells of the urether) and T24 (transitional cancer cells of the urine bladder) from ATCC. Bladder cancer cells were treated for 48 hours with 10nM DHT or not, with replacement after 24 hours. Expression of cell signaling proteins, was analyzed using Western Blot. Subcellular localization (cytoplasm, membrane, nuclear and cytoskeleton) of protein were studied using the ProteoExtract Subcellular Proteome Extraction Kit and Western blot analysis. We showed that DHT treatment significantly increased AR expression in bladder cell line HCV29. We also observed DHT-mediated activation of AKT/GSK-3β signaling pathway which play a central role in cancer progression. AKT leads to phosphorylation of the downstream mTOR targets Ribosomal protein S6 kinase, 70kDa, polypeptide 1 (p70 S6 kinase 1), Eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and subsequently activates protein synthesis by eIF4E factor. Our results also indicate, that expression of AR correlate with expression of EMT markers. We postulate that AR play crucial role in progression of bladder cancer and might be used as novel potential therapeutic target for treatment of bladder cancer. This work is supported by a grant from Ministry of Science and Higher Education through Jagiellonian University Medical College K/ZDS/006459.

No conflict of interest

A13. 3D cell architecture, a budding partner of extracellular vesicles production

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#Equal contribution of first authors

Introduction: The success of malignant tumours is conditioned by the intercellular communication between tumour cells and their microenvironment. To study the role of extracellular vesicles (EVs) as shuttles of information between cells, several in vivo and in vitro (2D and 3D) models have been proposed, however most of these studies rely on EV preparations obtained from cells growing in 2D. Unfortunately, 2D cultures poorly resemble the in vivo context. Knowing that 3D in vitro models mimic better tumour in vivo features, we hypothesized that EVs secreted by 3D cultures recapitulate better the signals involved in tumour intercellular communication, than EVs secreted by 2D cultures. Material and Methods: We performed a comparative analysis of biochemical features, small RNA and proteomic profiles of EVs secreted by 2D (monolayers) and 3D (spheroids) cultures of gastric cancer (GC) cells. We first established a 3D in vitro model of GC spheroids for isolation of EVs and characterized cell organization, polarization and viability by H&E, Ki-67, E-cadherin/Mucin-1 and AnV/PI staining. EVs were isolated from conditioned media of 2D and 3D cultures by differential ultracentrifugation and characterized by TEM, NTA and Imaging Flow Cytometry. Their small RNA and proteomic profiles were analyzed by NGS and LC-MS/MS, and validated by qRT-PCR and WB, respectively. Omics data were integrated using bioinformatics tools. Results and Discussion: Our 3D cultures recapitulated the histological properties of tumours and cell polarization often observed in vivo, and were more efficient in producing EVs than 2D cultures. From all small RNAs detected in EVs secreted by 2D and 3D cultures, microRNAs were the most abundant and each culture condition displayed a specific microRNA signature. The proteomic profile of EVs collected from 2D and 3D cultures was distinct, and a significant downregulation of the ARF6-associated signalling proteins was observed in 3D. Integrative network analysis of microRNA and protein data showed an overall upregulation of microRNAs and downregulation of proteins in 3D revealing a dynamic co-regulation in response to cellular architecture. Our study evidences a novel in vitro method to obtain EV preparations, which is more efficient and may be closer to the in vivo setting, comparing to the currently available options. Moreover, our work suggests that spatial cell organization influences the biogenesis and cargo of EVs, thus strengthening the biological relevance of 3D cultures for research on EVs.

No conflict of interest

A14. Pilot study of mTOR protein kinase inhibitor for melanoma cells proliferation and cell cycle.

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Introduction: Malignant melanoma is a disease with a high mortality rate due to rapid metastasis. Understanding melanoma signaling networks is the basis for molecular targeted therapy. Identification and pharmacological targeting of the correct network can lead to important clinical results. Material and Methods:

In this study we investigated the role of protein kinase inhibitors in cell proliferation using the BrdU ELISA test after 48-72h and signaling pathway in human melanoma cells. We studied the effect of protein kinase: PI3K, B-Raf, ERK1/2 and in human melanoma cells: WM793(VGP) and metastatic Lu1205(lung). Results and Discussion: Treatment of melanoma cell with protein kinase inhibitors led to significantly decreased cell proliferation. The most inhibition was noticed in case of applications a combination of mTOR inhibitors -Everolimus with PI3K kinase inhibitor -LY294002 which were reduced cell proliferation by: 62%. The use of a combination of inhibitors Everolimus and LY294002 gave noticeable effects on the level of cyclin D3, CDK6 kinase to about 80%. In contrast, the use of combinations of inhibitors U126 and LY294002 caused a similar decrease in the level of cyclin D1 and CDK4 kinase proteins. The level of the proteins of the cell cycle inhibitors increased upon application of each of the tested protein kinases inhibitors but the greatest increase observed following the use of mTOR inhibitors: Rapamycin and Everolimus about 70%, and GDC-0879 about 60%. In the present study on the effects of protein kinase inhibitors on the process of cell proliferation and cell cycle in melanoma cells, the most promising results were obtained with the combination of an inhibitor of the mTOR pathway -Everolimus with B-RAF kinase inhibitor GDC-0879 and Everolimus with inhibitor of ERK1 / 2 kinases -U126. This work was supported by a grant from Ministry of Science and Higher Education through Jagiellonian University Medical College K/ZDS/006458.

No conflict of interest

A15. Generation of CRISPR Cas9-mediated MSLN knockout ovarian cancer cell lines.

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Introduction: Mesothelin (MSLN) is a glycosylphosphatidylinositol-anchored cell-surface glycoprotein present on normal mesothelial cells and overexpressed in several human cancers, including ovarian cancer. The biological function of MSLN as well as its role in cancer is still unclear. The limited MSLN expression in normal tissues and high expression in ovarian cancer makes it an attractive candidate for study. Based on The Cancer Genome Atlas, elevated MSLN expression is associated with poor overall and early relapse- free survival. Additionally, our preliminary data indicate that MSLN is involved in invasion and peritoneal metastization upon stable downregulation in ovarian cancer cells. Here, we established an experimental model using CRISPR-Cas9 and lentiviral-based rescue for studying MSLN in ovarian cancer peritoneal carcinomatosis in more detail. Methods: We utilized a paired sgRNAs approach targeting the MSLN-encoding gene locus following PCR-based identification of Cas9 activity and homozygous knockout clones. Designed and aligned oligos were cloned into pSpCas9 (BB)-2A-GFP. After Cas9-activity test in HEK293T cells active constructs were transiently transfected into MSLN positive ovarian cancer cell lines following 72h incubation and GFP+ cell sorting, either for enrichment or single cell sorting into 96-well plates. Homozygous knockout clones were identified on genomic DNA by genotyping PCR and validated by DNA sequencing and Western blot. We also cloned the open reading frame of MSLN into pUltra for bicistronic expression of EGFP and MSLN lentivirally delivered to ΔMSLN cells. Stable EGFP+ cell lines were validated by flow cytometry and Western blot. Results: Cas9-activity test in HEK293T cells revealed expected band size for wildtype (1138bp) and ΔMSLN (899bp) in agarose gels. Despite lower transfection efficiency (<15%), sgRNAs delivered to ovarian cancer cell lines OVCAR3, OVCAR8 revealed Cas9-activity. Following single cell sorting we identified homozygous ΔMSLN clones fully depleted for MSLN and rescued by lentiviral transduction in ΔMSLN cells shown by Western blot (40 kDa) and flow cytometry (>95% positive cells). Conclusion: We successfully established MSLN knockout and rescued ovarian cancer cell lines. Established cell lines represent optimal tools allowing in-depth analysis of the metastatic function of MLSN in vitro and in vivo in ovarian cancer, the fifth leading cause of cancer death in women usually characterized by an extensive peritoneal carcinomatosis.

No conflict of interest

A16. Identification and Isolation of Prostate Cancer Stem Cells

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Introduction: Prostate cancer (PCa) is one of the most common malignancies in men worldwide and a leading cause of cancer-related mortality. PCa's phenotypical and functional heterogeneity has been associated with the presence of PCa stem cells (PCSCs) mingled with tumor bulk. PCSCs have been linked with high tumorigenicity and considered the driving force underlying progression, invasion and therapy resistance. Despite the great efforts to isolate and characterize PCSCs, a consensual and effective methodological approach is still lacking. Thus, the main goal of this study was to identify and isolate the potential PCSC population in PCa cell lines using three different approaches: "stem-cell" surface markers, autofluorescence, and ALDH activity. Materials and Methods: Five human PCa cell lines (LNCaP, VCaP, 22Rv1, DU145 and PC-3) were grown in adequate cell media. The percentage of intrinsic autofluorescent phenotype was assessed by firstly exposing the cells to riboflavin for cell population enrichment followed by flow cytometry analysis. A panel of "stem-cell" markers, including CD24, CD49b, CD49 and CD44 was also evaluated by flow cytometry. Additionally, ALDH activity was measured using ALDEFLUORTM Kit. PCSCs populations were separated from the tumor bulk cells by cell sorting and RT-qPCR was performed to assess mRNA levels of stem-cell associated genes. Results and Discussion: Overall, PCa cell lines revealed a homogeneous expression of all surface markers and no specific populations could be discriminated by flow cytometry. However, a higher staining intensity and transcript levels were found in more aggressive PCa cell lines, suggesting that their abundance in PCa cells is associated with malignant phenotype. Transcript levels of two pluripotency-associated genes, SOX2 and POU5F1, were also increased on these cells. Interestingly, a small population of putative CSCs was identified in VCaP and PC-3 cells by intrinsic intracellular autofluorescent assay. Nevertheless, a stem-like phenotype was only displayed by VCaP, which displayed high CD49, SOX2 and POU5F1 mRNA levels, and lower CD24 expression in fluo+ cells compared with fluo-. Surprisingly, 22Rv1 (negative control) also presented increased expression levels of the same markers. We are currently performing the final assays on potential PCSCs isolated by ALDH activity.

No conflict of interest

A17. Oncostatin M Induces Increased Invasiveness and Angiogenesis in Hepatic Cancer Cells through HIF1α-related release of VEGF-A

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Introduction: Oncostatin M (OSM), a pleiotropic cytokine belonging to the interleukin-6 (IL-6) family, can modulate hypoxia-dependent liver processes (development, regeneration and angiogenesis), contributing to chronic liver disease progression and hepatocellular carcinoma (HCC) development. Recently, hypoxia, as an independent signal operating through hypoxia-inducible factors (HIFs), has been shown to induce epithelial-to-mesenchymal transition (EMT) in cancer cells, including HepG2 cells. In this connection, OSM-related signaling pathway has been reported to up-regulate HIF1α and switch on EMT program. In this study we investigated in vivo and in vitro, the relationships between OSM, expression of vascular endothelial growth factor A (VEGF-A), and increased invasiveness. Materials and Methods. EMT, invasiveness, angiogenesis and signal transduction pathways were analysed by integrating morphological, molecular and cell biology techniques in the following experimental models: a) cohort of HCC patients b) HepG2 cells exposed to human recombinant OSM (hrOSM) or stably transfected in order to overexpress human OSM (H/OSM) or empty vector; c) murine xenograft. Results and Discussion. 1) Oncostatin M can induce EMT in both in vitro models (HepG2 cells exposed to human recombinant OSM or H/OSM) and stimulate invasiveness through VEGF release in culture medium and VEGF-dependent activation of PI-3K, ERK1/2, and p38MAPK signalling pathways; the use of specific pharmacological inhibitors against PI-3K, ERK1/2, p38MAPK signaling pathways as well as the use of neutralizing antibody raised against Flk-1(VEGF receptor type 2) or of a specific inhibitor of Flk-1 results in decrease of invasiveness induced by conditioned medium collected by HepG2 cells treated with hrOSM for 48hrs; 2) xenograft experiment shows an anti-proliferative effects of Oncostatin M, confirmed also in vitro (BrdU assay and cell cycle analysis); 3) oncostatin M seems to promote angiogenesis through OSM-dependent production of VEGF; 4) the highest levels of OSM transcripts correlate in HCC specimens with the highest rate of early tumor recurrence.

No conflict of interest

A18.

Selected for oral presentation – Symposium V

A19. Profiling the alternative splicing landscape of senescent cells

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Introduction: Cellular senescence, defined by an irreversible cell cycle arrest in response to potentially oncogenic stimuli, has been described as a protective mechanism in tumourigenesis and a therapeutic target in cancer. The senescence-associated secretory phenotype (SASP) is a pro-inflammatory response by senescent cells involving the release of cytokines, chemokines, growth factors and proteases that, in a cancer progression context, may be beneficial by the elimination of senescent cells or deleterious when triggering angiogenesis, cell proliferation and epithelial-to-mesenchymal transition. Despite senescence's importance in cancer and the suggested role of alternative splicing in its regulation, the transcriptional heterogeneity of senescent cells has, to our knowledge, been extensively characterised only at the gene expression level. Materials and Methods. Next-generation sequencing of RNA (RNA-seq) allows alternative splicing quantification with unprecedented precision. The inclusion level of an exon is commonly quantified by its percent-spliced-in (PSI) value, i.e. the proportion of RNA-seq reads providing evidence supporting its inclusion. However, a PSI ratio does not incorporate information about the number of reads used in the quantification of the cognate alternative splicing event, directly proportional to the precision of its estimate. Beta distributions can be exploited in modelling inclusion levels, using reads supporting exon inclusion and exclusion as surrogates of the distribution's shape parameters. We employed a computational pipeline, based on fitted beta distributions, to accurately quantify and compare alternative splicing across different types of senescent cells, relying both on

public and in-house RNA-seq datasets. Results and Discussion. Our analyses reproducibly identified, at a transcriptome-wide level, the alternative splicing changes specifically related with replicative and different types of induced senescence in multiple types of cells. For instance, Ras-induced senescence appears to associate with alterations in the splicing of genes involved in the secretory pathway and intracellular trafficking. Differential splicing analyses based on beta distribution modelling contribute to elucidate the specific alternative splicing signatures of different types of senescent cells, providing insights for targeting senescence in cancer therapies.

No conflict of interest

A20. The human Golgi anti-apoptotic protein induces cancer cell invasion by an H2O2-dependent mechanism

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Introduction: The severity of most cancer types is frequently related to their ability to spread and invade to other parts of the organism. The pharmacological approaches for oncological diseases rely mainly in anti-proliferative drugs and only few and poorly effective strategies are available to control cancer spread. Therefore, it is essential to understand the mechanisms involved in cancer cell spread so that new and effective therapeutic strategies can be developed. Human GAAP is a novel highly conserved Golgi cation channel that modulates Ca2+ fluxes from the intracellular stores, inhibits apoptosis and increases cell motility via store operated Ca2+ induced-calpain2 activation and focal adhesions turnover. Bioinformatics analyses suggest a link between dysregulation of hGAAP expression at the mRNA level and several human cancers. Significant upregulation of hGAAP has been detected in brain, lung, breast and prostate tumours. This work aims at exploring the role of the human Golgi anti-apoptotic protein (hGAAP) on cell and invasion. Materials and Methods, Results and Discussion: Unpublished data indicate that hGAAP overexpression in U2-OS cells increases in vitro (~80%) and in vivo (~80%) cell invasion measured by a matrigel-coated transwell and in vivo mouse invasion assays, respectively. Overexpression of hGAAP induces the extracellular proteolytic degradation of fluorescent gelatine and increases MMP2, but not MMP9 activity detected by a gelatine zymography. The overall intracellular levels of reactive oxygen species (ROS) (CellROX), as well of H2O2 specifically (HyPerRed), are significantly higher in cells overexpressing hGAAP. Conversely, the KD of hGAAP using siRNA results in a reduction of cell invasion, while overexpression of an hGAAP null mutant has no effect on cell invasion, proteolytic degradation or ROS levels. The reduction of both hGAAP-induced in vitro cell invasion and extracellular proteolytic degradation upon catalase treatment indicates that H2O2 plays a role in this mechanism. Although intimately associated, the mechanisms related to Ca2+ and ROS signalling involved in tumour cell migration/invasion are still not clear. A deeper understanding of hGAAP roles in cell biology might contribute to dissect this complex interplay and to provide new cancer biomarkers and/or druggable targets to be explored for anti-metastization therapeutic strategies.

No conflict of interest

A21. CELF2 at the core of a prognostic alternative splicing signature in colorectal cancer

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Introduction: Colorectal carcinoma (CRC) is a common malignancy, being the fourth cause of cancer-related deaths worldwide. Dysregulation of alternative splicing (AS) is a molecular hallmark of cancer, having been associated with initiation and development of CRC. However, the global patterns of dysregulation of AS and its association to prognosis in CRC remain largely unexplored. Materials and Methods: Clinically annotated tumour transcriptomes from The Cancer Genome Atlas (TCGA) were analysed in order to identify AS events with prognostic value in CRC. DNA methylation patterns in their vicinity were explored using TCGA methylation array data. A local CRC patient sample cohort and CRC cell lines were used in the experimental validation of the TCGA-derived AS prognostic signatures. Results and Discussion: We revealed a novel gene expression-independent AS signature, with prognostic value additional to that assigned to pathological stage and age, dominated by three AS events in the mRNA complement of CELF2, a gene encoding for RNA-binding proteins and reportedly an onco-suppressor in CRC. Those events relate to the expression of three isoforms with alternative promoter usage and potentially distinct sub-cellular localization and functions in RNA processing, namely AS regulation, mRNA edition and translation inhibition. We corroborated the prognostic value of alterations in CELF2 isoform expression using clinically annotated CRC samples from the local biobank. Further analyses in primary tumour-derived and metastasis-derived colon cancer cell lines confirmed those alterations as markers of increased tumour malignancy. Moreover, analyses of CRC TCGA DNA methylation profiles revealed significant differences in methylation in the vicinity of the three prognostic AS events in CELF2 associated with expression levels of the involved isoforms in matched patients. Our analyses suggest that a switch in the relative expression of CELF2 isoforms associates with prognosis in CRC. That switch dominates a gene expression-independent AS signature with prognostic value in CRC, representing a novel biomarker potentially usable in the prospective selection of patients for adjuvant therapy. We hypothesize that modifications in the dynamic balance between nuclear and cytoplasmic activities is the functional link between AS and the CELF2 prognostic value. This may be explained, at least in part, by local epigenetic alterations, given the observed changes in promoter methylation patterns in tumour samples from patients with poorer prognosis.

No conflict of interest

A22. Dual role of NRARP in Notch and Wnt signaling differentially impacts T-cell acute lymphoblastic leukemia

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy with a poor prognosis in patients with resistant or relapsed disease. Although NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available. We previously showed that loss of mir-181ab1 blocks NOTCH-induced T-ALL development partly by de-repressing the expression of NRARP (NOTCH regulated ankyrin repeat protein), a negative regulator of NOTCH signaling. These results suggested that de-regulation of NRARP expression may contribute to the pathogenesis of T-ALL. Here, we evaluated the role of NRARP in T-ALL. Materials and Methods: mRNA and protein expression were determined by quantitative PCR and western blot analyses. The functional evaluation of NRARP was performed in vitro and vivo upon NRARP overexpression in T-ALL cell lines and in the p53-null CD4-CD8- T-cell line D1, respectively. Results and discussion: We found NRARP significantly increased in T-ALL cells as compared to normal T-cell precursors, suggesting that NRARP is insufficient to block NOTCH oncogenic signals. To test this hypothesis, we overexpressed NRARP in human T-ALL cells. To our surprise, although NRARP overexpression blocks Notch signaling and delays the proliferation of T-ALL cells that display NOTCH1-activating mutations it promotes the expansion of NOTCH1-WT T-ALL cells. NRARP is known to stabilize LEF1, thereby contributing to Wnt signaling pathway activation. We found that NRARP overexpression down-regulates Wnt signaling in NOTCH1-mutated T-ALL cells whereas in NOTCH1-WT cells it potentiates Wnt signaling. Together these results suggested that

NRARP plays a dual role in T-ALL pathogenesis, regulating both Notch and Wnt pathways, with opposite functional effects depending on NOTCH1 mutational status and signaling levels. Consistent with this hypothesis, mice transplanted with D1 cells co-expressing NOTCH1 and NRARP developed leukemia significantly later than mice transplanted with D1-NOTCH1 cells. Importantly, mice transplanted with D1 cells overexpressing NRARP alone developed leukemia with similar kinetics to those transplanted with D1-NOTCH1 cells. Importantly, we validated this dual role of NRARP in human primary T-ALL cells. Our findings establish a new paradigm in what regards the outcomes of the crosstalk between Notch and Wnt signaling pathways in T-ALL, with important therapeutic implications.

No conflict of interest

A23.

Selected for oral presentation – Symposium IV

B1. Human epidermal growth factor receptor 2 in breast cancer: premilary study at Centro Hospitalar de Trás-os-Montes e Alto Douro

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Introduction: The human epidermal growth factor receptor 2 (HER2) plays an important role in the development of breast cancer and its accurate testing is extremely important. The amplification and/or protein overexpression of HER2, found in 20% of newly diagnosed breast cancers, is associated with a more aggressive clinical course and decreased survival time compared with tumors with normal levels of HER2. Material and Methods: Between August 2016 and March 2018, 65 patients with breast cancer were HER2 evaluated in the Centro Hospitalar Trás-os-Montes e Alto Douro. Fluorescence in situ Hybridization (FISH) technique in formalin-fixed paraffin-embedded cancer tissue specimens was applied to determine the HER2 status. FISH was performed using Zytovision dual-probe according to the manufacturer's protocol. Of the sample group, 16 patients with positive or equivocal HER2 were selected. Demographics, oncologic staging and long-term outcomes were reviewed. Results and Discussion: From a population of 65 patients, 12 presented HER2 amplified (18,5%), 4 patients were equivocal for HER gene amplification (6,2%) and 49 patients (75,4%) presented a negative result. All patients were female with median age of 61 years (Confidence Interval (CI) 95%, 53,92-67,96). Concerning tumour stage, 56,3% were stage I, 31,3% stage II and 12,5% stage III. From the 16 patients analysed, only 1 patient was hormonal receptor negative and 81,3% had Ki67 determination above 20%. 11 patients were submitted to surgery and 5 patients are under evaluation. 9 patients realized treatment with anti-HER2, with median time on treatment of 234,9 days (CI 95%, 134,2-335,58). The median of follow-up was 306 days (CI 95%, 184,36-428,02), all patients were alive at the end of follow-up and 9 patients without evidence of disease. Conclusion: Despite the reduced number of cases studied, the HER2 amplified by FISH incidence in our sample (24%) is accordingly to the literature. The treatment with anti-HER2 target therapy has shown impact in the overall survival of these patients. The accuracy of HER2 status determination has been essential for personalized cancer treatment, decreasing recurrence rates and improving survival.

No conflict of interest

B2. Uncovering the role of alternative splicing in Breast Cancer susceptibility

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Introduction: Recent genome-wide association studies (GWAS) have revealed the association of single nucleotide polymorphisms (SNPs) with breast cancer risk, although they fail to pinpoint the underlying biological mechanism for risk. Interestingly, most risk-associated SNP loci are located in non-coding regions, suggesting possible regulatory roles, such as altering the binding of transcription or splicing factors, as well as miRNAs. There has been a bias in the functional characterisation of GWAS loci towards the effect of regulatory

SNPs on transcription factor binding. Our aim is to determine the extent of the contribution of rSNPs influencing splicing among known breast cancer susceptibility loci. Material and Methods: We are screening GWAS variants for association with alternative splicing isoforms using bioinformatics tools such as sQTLseekR, on RNA-seq expression data from normal and tumoural breast samples, available from GTEx and TCGA projects, respectively. Our initial optimization of analyses has been carried on simulated data. We will posteriorly perform an in-vitro validation of the best candidates identified. Results and Discussion: Currently, we are performing statistical associations between genetic variations and splicing isoforms expression to find SNP-gene pairs associated with splicing isoforms levels. Our work will reveal the extent to which alternative splicing plays a role in breast cancer susceptibility, further contributing to the understanding of the biology behind this deadly disease.

No conflict of interest

B3. Revealing the role of allele-specific miRNA regulation in prostate cancer susceptibility

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Introduction: Prostate cancer (PCa) has saw its number of low-risk variants increasing dramatically in the last 10 years via genome-wide association studies (GWAS). However, the vast majority lie in non-coding regions, making it difficult to uncover the underlying molecular mechanisms. Increasing evidence supports a cis-regulatory role for most of the risk-associated SNPs, with the few functional studies carried in cancer GWAS loci mainly investigating the role of putative causal variants in altering transcription factor binding. Nevertheless, other cis-regulatory mechanisms, such as microRNA (miRNA) regulation, have been mostly overlooked. In the context of miRNA regulation, SNP alleles can either create or destroy target sites if located at the seed region of miRNAs or the target sequence of regulated genes, or regulate the levels of expression of the miRNAs themselves. Materials and Methods: We have previously developed a bioinformatic analysis pipeline for predicting allele-specific miRNA binding to cancer risk variants. Here, we selected the 217-haplotype tagging SNPs identified in PCa GWAS and queried their proxies in high linkage disequilibrium. These were then filtered based on location in either miRNA genes and/or miRNA target genes. Selected SNPs were then screened with the TargetScan miRNA-target-prediction algorithm, here adapted to analyse the impact of genetic variation on miRNA binding. Finally, results will be filtered for miRNAs with evidence of expression in prostate tissue and ranked according to previous evidence of cis expression quantitative trait loci (cis-eQTL), a proxy for cis-regulation. Results and Discussion: From the SNPs located at the 3' untranslated region of protein-coding genes, 25 were predicted to change the miRNA-mRNA binding stability in 13 genes, having three of these SNPs been previously identified as cis-eQTL. One of them, rs1058205 in KLK3, was already functionally validated to cause allele-specific binding of hsa-miR-3162-5p in another study, validating our approach. Interestingly, we also identified one variant mapping to a miRNA gene and we are currently adapting TargetScan to evaluate how this variant might be affecting the miRNA target genes. We believe our method (which includes crossing with cis-eQTL mapping in normal tissue) provides a new opportunity to quickly and systematically explore the role of miRNA cis-regulation in the risk loci identified not only for PCa, but for multiple common cancers.

No conflict of interest

B4. A complex karyotype in a B-cell chronic lymphocytic leukemia patient

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Introduction: B-cell chronic lymphocytic leukemia (B-CLL) accounts for 95% of all cases of CLL and is the most prevalent form of leukemia in adult individuals in the Western World, especially affecting the elderly. Classical and molecular cytogenetic analysis plays a very important role in the study of this pathology, since the detection of specific chromosomal alterations has prognostic and therapeutic implications. The anomalies associated with B-CLL are trisomy of chromosome 12 and deletions in loci located on chromosomes 6q21, 13q14, 11q22-q23 and 17p13. These anomalies are present in almost 80% of the cases. Material and methods: The authors present a case of a 80-year old women diagnosed with B-CLL and slight cognitive demency. She presented in 2017 with an history of axillar and retro auricular adenopathies with a cyclic growth of 5 months, stable lymphocytosis of 9080U/L without anemia or thrombocytopenia, elevated LDH 368U/L and multiple large adenopathies at physical exam (bilateral axillar and right inguinal with 5cm, bilateral supraclavicular). After 1 month she presented progressive enlargement of submandibular adenopathy, night sweets and increase of LDH. The cervical FNA cytology revealed a prolymphocytic transformation of CLL. She started chemotherapy with Chlorambucil + Prednisone but by appearance of new masses we changed to R-Bendamustine. At the moment she has fulfilled 5 cycles with good response and normalization of LDH. Cytogenetic analysis was performed before treatment. Cultures with 1,2-O-tetradecanoylphorbol-13-acetate (TPA) and with CpG-oligonucleotide DSP30 (DSP30)/Interleukin 2 (IL-2) were performed according to the laboratory protocols. Cytogenetic analysis followed the standard cytogenetic guidelines. Results and discussion: A complex karyotype was detected involving two abnormal cell lines: one with a translocation between the long arms of chromosomes 6 and 10 and a deletion of the short arm of chromosome 9 (10 metaphases), and the other with the same rearrangement plus a derivative of chromosome 1 with extra material on the long arm and a translocation involving chromosomes 7, 12 and 22 (2 metaphases).The detection of a complex karyotype is important to the prognostic and have therapeutic implications for the patient. Deletion of 6q is associated with the acceleration to PLL, a more aggressive stage of CLL. In our patient we detected a rearrangement between chromosomes 6 and 10 with possible disruption/loss of 6q22-23, compatible with the prolymphocytic transformation observed.

No conflict of interest

B5. APOC2 epimutation predicts a favorable prognosis in Acute Myeloid Leukemia

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Introduction: Acute myeloid leukemia (AML) is a highly heterogeneous hematological cancer characterized by a combination of genetic and epigenetic alterations. AML genomes present fewer mutations than other cancer types and multiple studies reporting mutations affecting epigenetic modulators and the prognostic value of methylation signatures have been recently published. Notwithstanding the recent developments, the main predictor of outcome remains AML cytogenetic karyotype refined by the identification of some gene mutations. Accordingly, patients can be stratified into favorable and poor prognostic risk groups that effectively benefit from adapted treatment strategies. The remaining 50% of patients belong to a heterogeneous intermediate-risk group for which clinical decision-making and outcome needs improvement. APOC2-encoded protein is involved in the lipid metabolism. It has been proposed as a potential biomarker detectable on serum of cancer patients but APOC2 epimutations and its prognostic value in AML were never

reported. In this context, our goal was to identify DNA methylation prognostic biomarkers in AML to improve patient risk stratification. Materials and Methods: APOC2 gene expression by RNA-seq and methylation by 450k array were assessed in the LAML cohort (n=200) from The Cancer Genome Atlas (TCGA). Patients in the LAML cohort were stratified into a favorable, intermediate or poor risk group by TCGA. Results and Discussion: The APOC2 gene was found to be differentially expressed in the three AML prognostic risk groups, being more expressed in the favorable group. Conversely, APOC2 was hypomethylated in the favorable risk group suggesting that demethylation of TSS200 and 5'UTR, first exon regions upregulates APOC2 expression. This hypothesis was corroborated by a negative correlation between APOC2 methylation and expression. In the intermediate group, both methylation and expression were more heterogeneous. Importantly, the overall survival of AML patients with APOC2 hypermethylation was significantly worse when compared with AML patients with a lower degree of methylation. In conclusion, these results demonstrate that the APOC2 methylation status can help predict the survival of AML patients and improve clinical decision-making. Future studies will focus on a possible role for APOC2 in AML pathogenesis and further assess the utility of APOC2 methylation as a prognostic biomarker and its use as a target for new therapies.

No conflict of interest

B6. Germline variants in DNA repair genes may underlie increased susceptibility for familial non-medullary thyroid carcinoma

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Introduction: The great majority of thyroid carcinomas derive from the thyroid follicular cells and are designated as "Non-Medullary Thyroid Carcinomas" (NMTC). NMTC may present as a familial form, being designated as FNMTC (Familial Non-Medullary Thyroid Carcinoma). In FNMTC families, patients frequently have thyroid cancer together with benign lesions (e.g., multinodular goiter). Although some susceptibility genes for FNMTC have already been identified (e.g., NKX2.2, FOXE1 and DICER1), these are mutated only in a small number of families. Therefore, the genetic basis of FNMTC remains largely unknown. Germline truncating mutations in DNA repair related genes (BRCA1, BRCA2, ATM, CHEK2 and MSH6) have been recently reported in cases with thyroid cancer, suggesting a role for these genes in FNMTC aetiology. Thus, the aim of this work was to analyse genes encoding proteins involved in DNA repair, in 48 families with FNMTC. Materials and methods: We selected 48 families with FNMTC of our cohort and searched for germline mutations through next generation sequencing (NGS) analysis, using the TruSight Cancer Kit (Illumina). This methodology allows the simultaneous analysis of 94 genes associated with cancer predisposition. NGS analysis was performed on leukocyte DNA from the probands of these families. The Illumina VariantStudio software was used for variant annotation. Genetic variants with allele frequency lower than 1% were selected, and to refine the bioinformatics analysis, their pathogenic potential was evaluated in silico (softwares SIFT, PolyPhen and MutationTaster). Results and discussion: In silico analysis of NGS data unveiled potentially pathogenic germline variants, in genes encoding proteins involved in DNA repair (CHEK2, BRIP1, PALB2, ERCC2 and ERCC4), which segregate with the disease in seven families. However, segregation studies are still ongoing for additional variants detected in this cohort. These preliminary results suggest that germline defects in DNA repair systems appear to be involved in the aetiology of FNMTC. These findings may have impact in the clinical management of FNMTC patients.

No conflict of interest

B7.

Selected for oral presentation – Symposium II

B8. Germiline Mutations in DNA repair genes may contribute to the susceptibility to multiple adenomas in colon and rectum

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Introduction: The number of individuals who develop multiple adenomas in the colon and rectum (MCRA) (10-99) but do not meet the clinical criteria for familial adenomatous polyposis, showing weak or even no family history of colon and rectal cancer (CCR), has increased in the last years. These patients often present an older age at diagnosis and constitute a clinically heterogeneous group with an increased risk for CRC development. Analysis of germline mutations in APC and MUTYH genes is recommended, according to international guidelines, however, results reported by our and other centers show that APC mutations are rare and in MUTYH are infrequent (≤20%). Considering the constant renewal of the colonic epithelium and the importance of DNA repair in this process, together with the known association of some repair genes with hereditary CRC syndromes (ex: MLH1, MSH2, MUTYH), the analysis of other DNA repair genes becomes relevant. In the present study, we aimed to identify the molecular cause(s) underlying the susceptibility to the development of MCRA. Materials and Methods: We included in this study 50 unrelated individuals with MCRA, without germline mutations in APC and MUTYH genes. We have analyzed germline mutations in 12 additional genes, known to be associated with the predisposition to CRC, namely for other CRC hereditary syndromes (MLH1, MSH2, MSH6, PMS2, EPCAM, PMS1, SMAD4, BMPR1A, PTEN, STK11, BUB1B, CHEK2), and in 34 genes encoding proteins involved in various DNA repair systems, associated with increased cancer risk. Next generation sequencing was performed using the TruSight Cancer panel in a Miseq platform. Results: Possibly pathogenic variants were detected in DNA mismatch repair genes (5/50, 10%)] and in homologous recombination and/or DNA Interstrand-Cross Link repair genes (11/50, 22%) among the 50 unrelated MCRA individuals. No possibly or likely pathogenic mutations were identified in genes associated to other polyposis syndromes. Conclusion: Germline defects in DNA repair systems appear to be important for the development of MCRA. Further studies are needed to confirm this association, which may have implications for the clinical management of these patients.

No conflict of interest

B9. Pan-cancer analysis of Centrosome Amplification uncovers its association with copy number alterations and poor clinical outcome

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Introduction: Centrosome amplification (CA) – the presence of more than two centrosomes in a mitotic cell – is a hallmark of cancer cells. Extra centrosomes are the main responsible for the generation of multipolar mitosis, and consequent genomic instability in cancer, and are associated with advanced tumour grade and poor clinical outcome. However, the molecular role of CA in tumourigenesis and its therapeutic value remain poorly understood. Materials and Methods: We have estimated relative CA levels across 9,721 tumour and 725 matched-normal samples belonging to 32 cancer types from The Cancer Genome Atlas using CA20, a published CA signature based on expression levels of 20 CA-associated genes, to investigate its association with different

tumour cellular and molecular features, as well as with available clinical information. CA20 was also employed to estimate CA levels in human cancer cell lines, for which both transcriptomic and drug-sensitivity profiles are publicly available, to identify compounds that could target cells with such abnormality. Results: We show that tumour samples have higher CA levels than matched-normal ones in all 15 cancer types with both sample types available. In breast cancer, CA is enriched in triple-negative tumours and we find an unprecedented association with both luminal B and invasive ductal tumours, when compared with luminal A and invasive lobular ones, respectively. This pan-cancer analysis extends the previous association between loss of p53 and CA to 10 different cancer types and reveals that CA is associated with higher mutation rate, somatic copy number alterations (CNA) and intra-tumour heterogeneity, three genomic instability features, together with lower stromal and immune cell infiltration. Linear regression modelling uncovers higher CNA and lower stromal cell infiltration as the main features independently associated with CA. Moreover, higher CA is associated with worse prognosis in 11 cancer types. Finally, correlation with drug activity highlighted some compounds as potential therapeutic options to selectively target cells with higher CA. Discussion: This work provides the first pan-cancer landscape of CA, suggesting that CA and CNA are concomitant genomic instability features. Our analyses show that CA is widely associated with poor clinical outcome in cancer and the candidate compounds identified herein can inspire the development of novel targeted cancer therapies.

No conflict of interest

B10. Uncovering the role of intronic cis-regulatory elements in CDH1 gene expression

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Introduction: Non-coding DNA regions comprise cis-regulatory elements (CREs) that are essential elements of cells genetic regulatory networks. As vital regulators of gene transcription, CREs have a profound impact in the normal gene expression. Hence, genetic variation of non-coding CREs in intronic regions within or nearby genes may explain disease etiologies. In this work, we are studying CDH1, a gene encoding E-cadherin, which is an essential protein for cell-cell adhesion. CDH1/E-cadherin dysregulation is associated with epithelial tumorigenesis, gastric cancer and cleft lip without cleft palate. However, its exonic mutations do not explain all mechanisms that lead to CDH1-associated disease phenotypes. Thus, this study aims to disclose the potential role of intronic CREs (iCREs) in the regulation of CDH1 expression and its role in a disease context. Material and Methods. Preliminary bioinformatic analyses on CDH1 locus mining ENCODE data for regions depicting epigenetic marks, as histone modifications, and other regulatory elements, as DNase hypersensitive sites and binding of transcription factors revealed two intronic sequences (iCRE 1 and iCRE8) as putative cis-regulatory elements. To ascertain functional relevance of CDH1 iCRE1 and iCRE8 elements, we deleted each separately in a gastric cancer cell line by using CRISPR-Cas9. We also deleted CDH1 exon2 as positive control. All cell lines engineered were purified by single-cell sorting to generate phenotypic homogeneous cell populations and the CRISPR-Cas9 deletions were confirmed by sequencing. Loss of function of CDH1 gene was assessed by Primer-extension assay and qRT-PCR, while E-cadherin expression pattern was determined by Imunocytochemistry and Western-Blotting. Results and Discussion. We obtained homogeneous clonal cell lines and fine-mapped deletions in the DNA sequence. The control exon2 homozygously deleted cell line showed total loss of CDH1 mRNA and protein. Heterozygous iCRE1 or iCRE8 CDH1-deleted clones presented monoallelic expression downregulation, overall decreased CDH1 mRNA expression and E-cadherin localization impairment. The overall

CDH1 expression phenotypes observed in iCRE1 or iCRE8 edited cells resemble CDH1-associated disease phenotypes. This supports the function of these intronic, non-coding sequences as cis-regulatory elements of CDH1/E-cadherin expression with a potential involvement in disease.

No conflict of interest

B11. An antisense transcript mediates MALAT1 response in human breast cancer

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Introduction: Long non-coding RNAs (lncRNAs) represent a substantial portion of the human transcriptome. LncRNAs present a very high cell-type/tissue specificity which has turned them good candidates for therapeutical applications during aging and disease. As example, targeting of MALAT1, a highly conserved lncRNA originally identified in human cancers, has shown promising results in several cancers progression. Still, the regulation and the cancer-type specificity of MALAT1 have not been directly addressed. MALAT1 locus is spanned by an antisense transcript named TALAM1. Here, we demonstrate that TALAM1 regulates MALAT1 levels during tumorigenesis in aggressive breast cancer, uncovering the complexity of MALAT1 regulation. Our biological characterization of TALAM1 reveals the functional properties of natural antisense transcripts in gene regulation and cancer aggressiveness, and raise new candidates for breast cancer targeting.

No conflict of interest

B12. Hist2h2ac regulates epithelial mesenchymal transition in mammary epithelial and breast cancer cells

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Introduction: Histone replacement affects gene expression regulating processes such as proliferation and differentiation. The histone H2A family is the most diverse including over 19 non-allelic variants and canonical histone isoforms. Even though there are studies showing severalH2A non-allelic variantsaltered in cancer, little is known about H2A canonical histone isoforms, which were, until recently, assumed to be DNA replication-dependent and functionally redundant. Material and Methods: We used HC11 mammary epithelial cells and T47D breast cancer cell linesto study the levels of EMT markers (E-cadherin, beta-catenin and Zeb-1) when Hist2h2ac was disrupted by shRNAs or over-expressed. Hist2h2ac mRNA levels were measured in undifferentiated HC11 cells with or without 10 ng/mL EGF for 24 h and FACS-sorted based CD24, CD29 and CD44 levels. Hist2h2ac expression was also studied using data from the Cancer Genome Atlas (TCGA). Results and Discussions: Hist2h2ac silencing in HC11 and T47-D cells inhibited EGF-induced Zeb-1 expression and E-cadherin down-regulation. This effect was reverted by Hist2h2ac re-expression, indicating that Hist2h2ac is necessary to allow epidermal growth factor-induced Zeb-1 expression and consequent E-cadherin down-regulation. Hist2h2ac mRNA was induced by EGF, specifically in the less differentiated CD24+/CD29+/CD44hi cell sub-population. Analysis of TCGA dataset showed that HIST2H2AC expression is altered in 17% breast cancers, being 16.8% of the cases related to gene amplification and/or mRNA upregulation. Conclusion: This is the first study that identifies a canonical histone isoform, Hist2h2ac, regulating the epithelial-mesenchymal process. Acknowledgements: This work was funded by Fundação para a Ciência e Tecnologia (FCT) project

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No conflict of interest

B13. The lncRNA NORAD correlates with aggressive breast cancer subtypes and confers protection from chemotherapy

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Introduction: The recently discovered human lncRNA NORAD is induced after DNA damage in a p53-dependent manner and plays a critical role in the maintenance of genomic stability. NORAD inactivation causes chromosomal instability and aneuploidy, which contributes to accumulation of genetic abnormalities and tumorigenesis. However, severe chromosome mis-segregations end up with cell death, suggesting that NORAD may act as an oncogene. p53, guardian of the genome, also plays an important role in response to stress conditions, inducing cell cycle arrest or apoptosis. In several types of cancer, including breast cancer, which is the most frequently diagnosed and the second with the highest mortality in women, higher NORAD expression is correlated with aggressiveness. Still, contradictory results from different studies impeded to unravel NORAD specific function in tumorigenesis. Here, using different breast cancer cell lines and antisense oligonucleotides for selective targeting of this lncRNA, we provide insights into the contribution of NORAD to aggressive breast cancer dynamics. Materials and methods: NORAD expression was determined in a set of human epithelial breast cancer cell lines (MCF-7, MDA-MB-231, 436 and 468) in comparison to a non-malignant human mammary epithelial cell line (MCF-10A) by RT-qPCR. Correlation between NORAD expression and survival of breast cancer patients was established through Kaplan-Meier Plotter. In order to unravel the role of this lncRNA, tumor-relevant phenotypes were analyzed after its knockdown using LNA GapmeRs and siRNAs, and after DNA damage induced by doxorubicin, in the MDA-MB-231 cell line. Cell viability was analyzed by alamarBlue® reduction assay, cell migration was analyzed by wound healing assay, cell apoptosis and cell cycle were analyzed by flow cytometry. p53 expression was determined by RT-qPCR and western blot. Results and discussion: NORAD expression is upregulated in breast cancer cells (MDA-MB-231, 436 and 468), which belong to the most aggressive subtypes (triple-negative and basal-like tumors), and is associated with poor prognosis. Moreover, NORAD knockdown inhibited proliferation, migration and sensitized the MDA-MB-231 cell line to chemotherapy through cell death possibly induced by p53, since its expression levels increase. Therefore, NORAD may represent a tumor marker for disease diagnosis, patient prognosis or therapy response, and may represent a therapeutic target in breast cancer.

No conflict of interest

B14. Identification of potential miRNAs biomarkers for high-grade prostate cancer by integrated bioinformatics analysis

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Introduction: Several miRNAs have been proposed as biomarkers for prostate cancer (PCa) management. The increasing number of datasets available in the Gene Expression Omnibus database offers a new approach to identify the miRNAs related to high-grade PCa (Gleason score ≥ 7). Bioinformatics studies provide scientists with some criteria to hierarchize trials to later validate in vitro the predicted networks and discover novel

biomarkers. The aim of our study was to suggest a miRNAs profile with utility in PCa detection and prognosis using bioinformatics tools. Material and Method: Three mRNA datasets (GSE26022, GSE30521, GSE46602) were selected to identify the differentially expressed genes (DEGs) in high-grade PCa. Furthermore, two miRNA datasets (GSE45604, GSE46738) were analyzed to select the differentially expressed miRNAs (DEMs). GEO2R was used to compare DEGs and DEMs between normal controls and high-grade PCa. Functional and pathway enrichment analysis was performed for the predicted targets using DAVID database. A p value <0.05 was used as threshold value. Protein–protein interaction (PPI) network was established by STRING, considering a confidence score ≥0.7 as threshold value. Results and discussion: A total of 973 DEGs were identified after the analyses of the mRNA datasets. The 617 down-regulated genes were mainly enriched in angiogenesis, hemidesmosome assembly, negative regulation of epithelial cell proliferation and cell adhesion, while the 356 up-regulated genes were mainly enriched in extracellular matrix organization, protein transport, oxidation-reduction process and cell division. Furthermore, among these DEGs, we selected in the PPI network analysis 10 hub genes, which were mainly enriched in the biological processes of negative regulation of apoptosis and positive regulation of cell migration. The most significant KEGG Pathway was PI3K-Akt signaling pathway, which regulates diverse cellular functions, among them growth, proliferation, and survival. A double way to select the most relevant miRNAs was assayed. Eighty one DEMs were sorted out from the GSE45604 and GSE46738 datasets. On the other hand, 165 miRNAs were predicted to be hub genes regulators. Finally, we identified 30 miRNAs common to both procedures. Twelve of these miRNAs (miR-1, -365, -132,-133a, -133b, -195,-200c, -339-5p, -222, -21, -221 -708) act as regulators of two or more hub genes identified in our study. Those miRNAs are involved in several steps of PCa progression and have been associated mainly with aggressive PCa, poor survival and resistance to treatment. We suggested that this miRNA signature may be useful for high-risk PCa stratification and management.

No conflict of interest

B15. Identification of risk variants for breast cancer: a comprehensive analysis of RNA-Seq data

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Introduction: To better understand breast cancer risk, it is crucial to identify common variants like single nucleotide polymorphisms (SNPs) with regulatory potential, as these often modulate allelic expression (AE) levels. Therefore, an accurate quantification of AE is imperative. High-throughput RNA sequencing (RNA-seq) provide not only bulk expression quantification but also single nucleotide variant counts, ideal to quantify AE. However, RNA-seq data analysis has to be tailored specifically for the purpose of quantifying AE. To date computational analysis of such data is challenging due to the intrinsic complexity of the transcriptome. Since no single RNA-seq analysis pipeline can be applied to all cases, this work intends to assemble the best tools for precise AE quantification using RNA-seq data. Materials and Methods: We performed RNA-seq on 27 samples extracted from normal breast tissue, 12 from healthy controls and 15 from breast cancer patients. For each sample, around 112M paired-end reads of 100bp were obtained. Initial quality assessment of the data was performed with FastQC. Subsequently, data from three samples were used to compare the performance of seven trimming tools, focusing mainly on the per base quality score and presence of overrepresented sequences. These samples were chosen based on the quality of raw data for those features, namely one was of very good, one of reasonable and one of poor quality. The first comparison included running times, memory usage and number of trimmed reads. We are currently conducting the alignments with GSNAP, the top SNP-aware aligner. Variant calling will be performed with three well-documented tools and SNPs identified with all variant callers will be given priority for further analysis. Results and Discussion: Overall, sequencing results exhibited very good quality, respecting GC content, per base quality score and overrepresented sequences, among other features. Initial comparison of trimming tools showed that one performed better when considering time/memory usage, and another regarding the number of trimmed reads. Following read mapping, other metrics will be considered to assess trimming and alignment qualities. The present study

provides a pipeline for an accurate genetic variant detection from RNA-seq data. This will improve the detection of variants with differential AE and mapping of potential regulatory SNPs in samples from breast cancer cases and controls.

No conflict of interest

B16. Differential molecular signature in patients from African origin with Triple-Negative Breast Cancer

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Introduction: Women of Black race (BW) are prone to have more aggressive forms of breast cancer (BC). These women are usually pre-menopausal, have worse prognosis and higher mortality rate when compared with patients from other races, even when socioeconomic factors are accounted for. Triple-negative breast cancer (TNBC), the most aggressive form of BC, is more frequently diagnosed in young BW. TNBC is clinically "negatively defined", lacking oestrogen and progesterone receptor expression, and amplification of HER2. Thus, TNBC does not have any known therapeutic targets and, in most cases, shows resistance to conventional therapies. Materials and Methods: To identify the driving biological factors of this racial disparity, we performed a comprehensive differential gene expression (DGE) analysis using RNA-sequencing BC data from The Cancer Genome Atlas. In a total of 1097 BC patients, 183 are BW patients, from which 32 have TNBC (17.49% of all BW); 757 are of White race (WW), of which 69 have TNBC (9.11%); and 61 are Asian (AW), of which 8 have TNBC (13.11%). DGE was performed using the R package edgeR, applying the pipeline proposed by SARTools, another R package, which provides tools to generate descriptive and diagnostic graphs of the DGE. Results and discussion: The first step of the analysis, i.e. to determine DGE between BW with TNBC and TNBC patients of other races, revealed 251 upregulated and 269 downregulated genes (adjusted p-value < 0.05, |log2 (Fold Change)|>1), applied in all the analysis). The second step was to remove genes associated with race alone, and not with TNBC in BW per se. For that we performed a DGE analysis between all BC cases in BW and all BC cases in the other races, resulting in 364 upregulated genes and 138 downregulated genes in BC of BW. Then, common genes between the two analyses were identified and extracted from the previous list, resulting in 143 upregulated and 242 downregulated genes, exclusively differentially expressed in BW with TNBC. We are currently studying the biological relevance of these genes in TNBC development and patients' clinical data, which includes gene-set enrichment analysis to identify pathways particularly important in BW-TNBC progression and clinical implications.Our work will unveil the molecular signature(s) that characterise and define molecularly the aggressive form of BC in BW and, ultimately, which will guide the development of new therapeutics for this unmet medical problem in BC care.

No conflict of interest

B17. Integrative genomic approach elucidates the risk mechanism for breast cancer associated 5q14.1 locus

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Introduction: Genome-wide association studies (GWAS) have identified a significant number of new loci associated with risk for developing breast cancer (BC). Follow-up functional studies of these loci, including previous evidence from our work, have demonstrated that cis-acting regulatory variants (rSNPs) are strongly involved in BC risk. Differential allelic expression (AE) is a hallmark of cis-regulation. Therefore, we hypothesize that mapping the cis-regulatory loci in normal breast tissue, using AE analysis, is the most efficient way to map the causal variants of risk. Material and Methods: We began our analysis by performing whole-genome AE quantification in 64 normal breast tissue samples using commercial arrays, followed by AE mapping analysis. Then, we overlapped our cis-regulatory variants map with published BC GWAS variants (r2>0.8). Finally we selected one BC risk locus to perform in-silico functional analysis to identify the underlying regulatory mechanism. Results and discussion: We found that 76.1% of all expressed genes display differential AE, indicating that cis-regulation is a widespread mechanism in breast tissue. For one-sixth of these genes we were able to identify common cis-variants possibly affecting gene expression levels (rSNPs) (10% FDR significance). Overlapping of our rSNP candidates with GWAS loci allowed us to derive a list of targeted genes and candidate regulatory risk variants for nine risk loci, including 5q14.1 previously reported as associated in GWASes meta-analyses. At this locus, we identified three functional rSNPs, one located at the promoter of ATG10 and one located in the shared promoter of RPS23 and ATP6AP1L genes, whose minor alleles correlate with higher expression of both ATG10 and ATP6AP1L and with lower expression of RPS23. The minor alleles of these two variants, in phase with the GWAS-SNP protective allele, confer differential binding of polymerase II and cMYC, thereby influencing the target genes' expression. Additionally, we identified one rSNP with potential to be affecting ATG10 splice variants levels. Overall, our results suggest that variants in the 5q14.1 locus associated with BC risk, act by regulating ATG10, RPS23 and ATP6AP1L expression, through regulation of transcription factor binding and splice variants expression. This study reveals that AE mapping is a powerful method to identify variants with direct impact in complex regulatory landscapes, such as that of the 5q14.1 risk locus.

No conflict of interest

B18. Identifying novel genes associated with Breast Cancer susceptibility using Allelic Expression ratios

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Introduction: Breast Cancer (BC) is the most common cancer among women worldwide. However, the current knowledge of BC susceptibility only accounts for half of the familial cases. The few functional studies performed for genome-wide association studies (GWAS) loci revealed a role for cis-regulatory variation, suggesting that instead of altering protein function or structure, the genetic risk variants may be acting by regulating gene expression levels. Therefore, we hypothesise that the most efficient approach to tackle BC missing heritability is to focus susceptibility studies on variants that show greater cis-regulatory potential. Hereby we present an innovative approach to genetic association studies using a quantifiable readout of the effect of cis-regulatory variants — differential allelic expression (dAE). Materials and Methods. To select candidate genes to perform association studies using dAE, we carried differential expression analyses in 144 normal matched and 1992 breast tumours samples from the METABRIC project. We compared expression between normal-matched and tumour tissue, and also between different BC clinical variables for tumour tissue (like oestrogen receptor status and tumour grade). We also filtered the analysed genes for known cis-regulation in normal breast tissue (AE data from our lab). Finally, we compared our list of differential expressed genes with genes which demonstrated association in the first phase of BC GWASes, but lacked replication studies. Results and Discussion. We identified 1029 genes both under cis-regulation and differentially expressed in the tested conditions (FDR $\leqslant$ 1% and absolute fold-change $\geqslant$1.5). From this list, 13 genes have been already associated with BC risk (p≤5x10-8). Only one gene NR3C2, which is downregulated in tumour tissue when compared to normal-matched (adjusted p-value = 1.897142e-121, log2(FC)= -1.0136), was associated in the GWAS first stage but not in the replication dataset and therefore, fulfil all criteria previously explained. We will next perform case-control association studies using AE ratios from BC cases (normal-

matched tissue) and controls (normal breast), for this and other candidate genes. Using this novel approach we expect to identify novel loci that confer risk to BC and that also contribute to disease progression, as well establish the importance of cis-regulation in BC.

No conflict of interest

C1. Modulation of Sodium Iodide Symporter expression by tumor-associated signaling pathways

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Introduction: Expression of the Sodium Iodide Symporter (NIS) in thyroid cancers (TC), responsible for iodide uptake, allows the use of radioactive iodine (RAI) as treatment of choice for TC metastatic disease. Still, about 30% of patients with metastatic TC lose the ability to respond to RAI therapy, which reduces their survival rates. Thus, novel approaches to enhance iodide uptake, with the goal of maximizing RAI therapy efficiency in TC are clinically relevant. Regulation of NIS expression in the thyroid gland depends primarily on signaling induced by the Thyroid-Stimulating Hormone. However, several other pathways have been proposed to also influence NIS expression in thyroid cells. The PI3K/mTOR and MAPK pathways have been associated with NIS downregulation whereas RAC1-mediated induction of p38 kinase activity was shown to enhance TSH-induced NIS expression. Interestingly, our preliminary data, gathered from TC samples, indicates the existence of an inverse correlation between NIS expression levels and the overexpression of RAC1b, a hyperactive RAC1 splicing variant. Currently, we are further investigating the impact of RAC1/RAC1b signalling on NIS expression levels and how these crosstalk with other pro-tumorigenic pathways in different genetic backgrounds. Materials and Methods: Normal and thyroid cancer cell lines were used to access NIS expression in the presence and absence of RAC1b exogenous expression, and upon treatment with inhibitors targeting RAC1, PI3K/mTOR and MAPK pathways. NIS expression levels were analyzed by qPCR. Results and Discussion: Consistent with our preliminary findings, RAC1b overexpression in PCCL3 normal thyroid cells induced a clear decrease in NIS expression levels. Furthermore, treatment with the RAC1 inhibitor EHT1864, consistently decreased NIS transcript levels, supporting the association between RAC1-signaling and NIS transcriptional regulation. Additionally, both LY294002 and Selumetinib (PI3K and MEK inhibitors, respectively) upregulated NIS transcript levels in PCCL3 cells. In thyroid cancer context, the impact on NIS expression of modulating these pathways varied among the different thyroid cancer cell lines tested, possibly due to different genetic and signaling contexts. These preliminary results support the relevance of further studying the impact of modulating RAC1, PI3K/mTOR and MAPK pathways on NIS expression, as a potential approach to enhance the efficiency of iodide uptake.

No conflict of interest

C2. STAT5 is Essential for IL-7-Mediated Viability, Growth and Proliferation of Human T-Cell Acute Lymphoblastic Leukemia Cells

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) constitutes an aggressive childhood malignancy. Whereas interleukin-7 (IL-7) is essential for normal T-cell development, it can also accelerate T-ALL development in vivo and leukemia cell survival and proliferation by activating PI3K/Akt/mTOR signaling. Here, we investigated whether another key pathway, JAK/STAT, could also mediate IL-7 T-ALL-promoting effects. Materials and Methods. Cellular models used in this study included TAIL7, an IL-7 dependent T-ALL cell line; HPB-ALL, an IL-7 responsive T-ALL cell line; primary T-ALL samples collected at diagnosis; and patient-derived xenografts (PDX). Cells were stimulated or not with IL-7 in the presence or absence of inhibitors or after genetic manipulation. In the latter case, transient electroporation of plasmids, or retroviral and lentiviral transductions were performed. STAT5 was inactivated using a small molecule inhibitor and AZD1208 was used as pan-PIM inhibitor. Flow cytometry, western blot, 3H-thymidine incorporation, qPCR, RNA-seq and STAT5 ChIP-seq were used to characterize different levels of impact of IL-7 on T-ALL cells. Results and Discussion. We show that IL-7 induces JAK/STAT pathway activation in T-ALL cells and that STAT5 inactivation prevents IL-7-mediated T-ALL cell viability, growth and proliferation. At the molecular level, STAT5 is required for IL-7-induced downregulation of p27kip1, and upregulation of the transferrin receptor, CD71. Surprisingly, STAT5 inhibition does not significantly affect IL-7-mediated Bcl-2 upregulation, suggesting that, contrary to normal T-cells, STAT5 promotes leukemia cell survival through a Bcl-2-independent mechanism. STAT5 ChIP-seq and RNA-seq reveal a diverse IL-7-driven STAT5-dependent transcriptional program in T-ALL cells, which includes BCL6 inactivation by alternative splicing, and upregulation of the oncogenic serine/threonine kinase PIM1. Pharmacological inhibition of PIM1 abrogates IL-7-mediated proliferation on T-ALL cells, indicating that strategies involving the use of PIM kinase small molecule inhibitors may have therapeutic potential against a majority of leukemias that rely on IL-7R signaling. Overall, our results demonstrate that STAT5, in part by upregulating PIM1 activity, plays a major role in mediating the leukemia-promoting effects of IL-7/IL-7R.

No conflict of interest

C3. Upregulation of RAC1/PAK1 signalling promotes DNA damage repair in colorectal cancer cells

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Introduction: Colorectal cancer is one of the most prevalent types of cancer worldwide. The GTPase RAC1 and its effector PAK1 have been found overexpressed or hyperactivated in this type of cancers, particularly those with more aggressive and invasive features, which is frequently correlated with resistance to chemotherapeutics and unfavourable clinical prognosis. Previously, we described a new signalling pathway in which activation of RAC1/PAK1 signalling promotes a transcriptional switch between the BCL6 repressor and the STAT5 transcriptional activator at a restricted subset of gene promoters. Materials and Methods: Here we used a novel combinatory ChIP-Seq approach for the genome-wide identification of the BCL6/STAT5-switch target genes. Results and Discussion: Ontological enrichment analysis among the identified target genes revealed an overrepresentation of genes involved in DNA damage repair. Using the comet assay as a read out for the extent of DNA damage, we show that the activation of RAC1/PAK1 signalling provides partial protection

against damage induced by alkylating agents and significantly accelerates DNA damage repair. This work highlights an additional role for the RAC1/PAK1 signalling axis that may contribute to the chemoresistant phenotype of aggressive colorectal tumours.

No conflict of interest

C4. Uncovering the role of acetate in the crosstalk between monocarboxylate transporters and oncogene signalling pathways in CRC

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Introduction: Colorectal cancer (CRC) is one of most commonly diagnosed cancers worldwide. The normal intestine microbiome harbours hundreds of different bacterial species which play several roles in human health, such as protection against pathogens, immune system maturation, degradation of toxic substances, digestion of complex carbohydrates and production of short-chain fatty acids (SCFAs). SCFAs, specifically acetate, propionate and butyrate are produced by propionibacteria and constitute a major source of energy for colonocytes. Previous reports from our group showed that acetate inhibits CRC cell proliferation, induces apoptosis, promotes lysosomal membrane permeabilization, increases CRC cell glycolytic phenotype and regulate its own uptake by increasing the expression of monocarboxylate transporters (MCTs). However, the signalling pathways associated to the phenotypic changes induced by acetate have not been characterized. In order to clarify this issue, here we aimed to evaluate the involvement of acetate in the expression levels of oncogene signalling pathways molecules known to be important in CRC cells survival namely PI3K/AKT and MAPK pathways. We also aimed to uncover the role of MCTs in the regulation of those signalling pathways in CRC cells exposed to acetate. Our data suggest that acetate treatment is able to modulate the expression levels of some signalling molecules, namely phosphorylated cRAF and ERK, in a time and dose-dependent manner. Moreover, preliminary results herein presented show that acetate increases the expression levels of MCT-1 and MCT-4, in order to maintain the uptake of SCFAs. To the best of our knowledge this is the first work studying the interplay between an intestinal microbiome product acetate and two important hallmarks of cancer, namely oncogene signalling activation and metabolism in CRC. This study might help in the discovery of new approaches in prevention/therapy of CRC.

No conflict of interest

C5.

Selected for oral presentation – Symposium I

C6. Regulation of the Alternative Splicing of Tumor-Related RAC1b by Signal Transduction Pathways

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Introduction: In colon cancer distinct genetic subtypes have been described, one of which involves overexpression of RAC1b, a variant generated by alternative splicing. Aberrant splicing is known to occur in cancer and can be caused by mutation in a gene or splicing factor but also represents a dynamic response to oncogene-induced cellular signaling and in this case it may be pharmacologically targeted. Here we explore how signaling pathways are involved in the deregulation of alternative RAC1b splicing in colorectal tumor cells. Materials and Methods: HT29 colorectal cells represent serrated colorectal tumors with BRAF gene mutation V600E in one allele and RAC1b overexpression. Cells were transfected with shRNA vectors directed against

target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24h later by Western Blot and qRT-PCR. Treatment with kinase inhibitors or anti-inflammatory drugs was performed 24h prior to cell lysis. Results and Discussion: Two kinases, SRPK1 and GSK3β, were found required to sustain RAC1b levels and both were shown to act upon the phosphorylation of splicing factor SRSF1, which binds to and promotes the inclusion of the alternative exon in RAC1b. SRPK1 knockdown or pharmacological inhibition reduced SRSF1 phosphorylation decreasing its nuclear translocation and concomitantly RAC1b splicing. The same regulatory pathway was also found to be controlled by GSK3β. Interestingly, GSK3β phosphorylation was identified to serve as target for the anti-inflammatory drug ibuprofen, which inhibits RAC1b overexpression. Together, our results demonstrate that oncogenic signal transduction pathways deregulate alternative splicing and this may be drug revertable.

No conflict of interest

C7. Unravelling ErbB2 Glycosylation Signature in Gastric Cancer Cells

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Introduction: The abnormal expression and activation of the human epidermal growth factor receptor 2 (ErbB2) represent central molecular events underlying the neoplastic transformation of the gastric tissue [1]. Despite the extracellular domain of this cancer-relevant receptor tyrosine kinase (RTK) being a well-known target for extensive glycosylation, its detailed glycosylation profile and the molecular mechanisms through which it actively tunes ErbB2 towards malignancy in gastric cancer (GC) cells remain elusive [2, 3]. Materials and Methods: The expression of relevant glycosyltransferase-coding genes, and the expression and activation of the ErbB receptors were assessed in four GC cell lines. Further glycan characterization was performed in NCI-N87 GC cells, an in vitro model of ErbB2 overexpression and hyperactivation. Using NCI-N87 whole cell lysates, ErbB2 was immunoprecipitated and validated by MALDI/TOF-TOF tandem mass spectrometry. Receptor's glycosylation was confirmed through Peptide-N-Glycosidase F digestion and characterized using a panel of carbohydrate-binding lectins and monoclonal antibodies (mAbs). The expression of genes controlling the biosynthesis of cancer-associated glycans in association to ErbB2 status were studied. Expression and activation of ErbB2 were assessed in ErbB2-overexpressing cells submitted to in vitro deglycosylation and mAb-mediated glycan blocking. Results and Discussion: Cellular- and receptor-specific glycan profiling of ErbB2-overexpressing NCI-N87 cells disclosed an intricate glycosylation pattern harboring the tumor-associated sialyl Lewis a (SLea) antigen. The expression of SLea and key enzymes integrating its biosynthetic pathway showed to be strongly upregulated in this GC cell line. An association between the expression of ERBB2 and FUT3, a central gene in SLea biosynthesis, was additionally established in GC patients. Furthermore, cellular deglycosylation and CA 19.9 antibody-mediated blocking of SLea drastically disrupted both receptor's expression and activation in NCI-N87 cells. Conclusion: Our results show that the disclosed glycosylation profile of ErbB2 in GC cells has a major functional impact on receptor's biology with potential clinical applications. Furthermore, NCI-N87 cell model constitutes an appealing in vitro system to study glycan-mediated regulation of ErbB2 in GC [4].

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D1. Effect of physical exercise on prostate cancer in Wistar rats: an ultrasonographic approach

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Introduction: Prostate cancer (PC) is one of the most common tumors in men. The biological mechanisms underlying the effects of physical exercise in cancer are not fully elucidated. Ultrasonography is one of the techniques for monitoring prostate gland. This work intended to assess the effects of physical exercise in prostate dimensions in a rodent model of prostate cancer. Materials and methods: Forty-one male rats Wistar Unilever with four weeks of age were randomly assigned to four experimental groups: group I (control sedentary, n=10), group II (control exercised, n=10), group III (PC-induced sedentary, n=10) and group IV (PC-induced exercised, n=11). Animals from exercised groups were trained on a treadmill, since eight weeks of age. Induction of prostate cancer started at 12 weeks of age and was achieved through sequential administration of flutamide, testosterone propionate, N-methyl-N-nitrosureia (MNU) and subcutaneous implants of testosterone. The rat prostate was evaluated by ultrasonography at 12, 15, 21, 32 and 35 weeks of age. A complete transverse scan of the ventrolateral and dorsal prostate lobes was performed. The radius of the prostate lobes was measured using the callipers of the ultrasound apparatus. Ventrolateral and dorsal prostate area resulted from the sum of the left and right lobes area. Results and Discussion: Three animals died before the end of the experimental protocol, two animals from group I and one from group IV. In the first and second ultrasonographic examinations, the prostate volume was similar among groups ($p>0.05$). In the third exam, the group III presented a higher volume of ventrolateral prostate lobe when compared with group I (0.64±0.10cm2 versus 0.48±0.07cm2). The main significant difference between groups occurred in the fourth ultrasonographic examination between group I (0.48±0.12cm2), and groups III (1.78±0.94 cm2) and IV (1.43 ± 0.28 cm2). In the last exam, the area of ventrolateral lobe was significantly different between groups I and IV (0.34±0.15 cm2 versus 0.66±0.31cm2). The dorsal prostate was only observed in the last two ultrasonographic examinations. In the last exam, the area of dorsal prostate was lower in group I (0.28±0.08cm2) when compared with groups II, III and IV (0.54±0.23cm2, 0.47±0.18cm2 and 0.49±0.19cm2, respectively) ($p<0.05$). Conclusions: Physical exercise did not affect prostate size. Ultrasonography allowed the detection of variations of prostate volume during the experiment, being an excellent tool for monitoring prostate carcinogenesis.

No conflict of interest

D2. The endocrine disruptor methoxychlor modulates the apoptotic pathways and glycolytic metabolism in human prostate cancer cells

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Introduction: The reprogramming of cancer cell metabolism, recognized as a hallmark of cancer, includes a shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis, which culminates in an increased lactate production. We and others previous work have shown that steroid hormones play a role driven the metabolic changes associated with prostate cancer (PCa) progression. Nevertheless, the panoply of metabolic (de)regulators linked with PCa remains poorly known. Endocrine disruptors interfere with the synthesis and mechanism of action of natural hormones, and have also been implicated in carcinogenesis. Methoxychlor (MXC) is a pesticide widely dispersed in the environment and several reports have showed its estrogenic properties. However, the MXC effects inducing metabolic alterations in prostate cells are largely unknown. This study aimed to analyze the effect of MXC on cell viability, apoptosis and glycolytic metabolism of neoplastic (LNCaP and PC3) and non-neoplastic (PNT1A) human prostate cells. Materials and Methods: PNT1A, LNCaP, and PC3 cells were cultured in the presence or absence of a range of MXC concentrations (0.1-100 μM) for 48 and 72 hours. Protein expression and activity of target modulators of apoptosis and glycolytic metabolism were assessed by means of Western blot analysis and biochemical assays. Results and Discussion: The obtained results showed that MXC diminished the viability of both neoplastic and non-neoplastic prostate cells in a time- and concentration-dependent manner. Moreover, MXC-treatment decreased the apoptotic rate of PNT1A cells, despite the observed decrease in cell proliferation. Curiously, in LNCaP- and PC3-treated cells MXC had an opposite effect increasing caspase-3 activity and up-regulating the expression of apoptotic regulators. Concerning cell metabolism, the obtained findings showed that MXC stimulated the glycolytic flux in both non-neoplastic and neoplastic human prostate cell lines, as indicated by the enhanced glucose consumption and lactate production. This metabolic response was supported by the increased expression of glucose transporters and activity of glycolytic enzymes, such as lactate dehydrogenase. In conclusion, MXC may have a role in the development and progression of PCa by suppressing apoptosis in non-neoplastic prostate cells and stimulating the glycolytic pathway in both non-neoplastic and neoplastic cells.

No conflict of interest

D3. Mutant IL-7 receptor cooperates with Myc and activated Notch to accelerate T-cell leukemogenesis in zebrafish

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological cancer with high prevalence in children. More than 70% of human T-ALL samples proliferate in response to interleukin-7 (IL-7), a cytokine produced in the leukemia milieu, while around 10% of T-ALL patients display oncogenic gain-of-function mutations in the interleukin-7 receptor (IL-7R) gene. These mutations lead to constitutive activation of IL-7R-mediated signaling that promotes cell transformation. However, it remains to be determined whether aberrant IL-7R-mediated signaling is sufficient to trigger leukemia in developing T-cell precursors, and which oncogenes may cooperate with IL-7R activation during the leukemogenic process. We propose to tackle this issue using zebrafish transgenic models. Materials and Methods. Transgenic zebrafish were generated

harboring patient-specific mutations in IL7R, alone or in combination with well-established drivers of T-ALL, such as MYC, NOTCH, TAL1, LMO2 or HOXA. These transgenic fish were subsequently characterized for disease onset and progression. The mutant IL7R alone group was compared to the different combination groups using the log-rank test and Kaplan-Meier analysis. Differences in the overall numbers of leukemia-propagating cells (LPCs) were also assessed by performing limiting dilution transplantation assays of primary tumors into secondary recipient fish. Fluorescence-positive and PI-negative (live) cells were collected and isolated by flow cytometry and injected at different leukemia cell dilutions (10,000 down to 10 cells). Fish were examined for up to 90 days and the number of positive fish recorded. Data were uploaded into the web-based ELDA (Extreme Limiting Dilution Analysis) statistical software to determine the frequency of LPCs.Results and Discussion. Our initial observations suggest that mutant IL7R alone is not capable of inducing leukemia in the zebrafish. However, IL7R mutations collaborate with two well-known drivers of T-ALL, Myc and Notch, to accelerate T-ALL onset. Limiting-dilution cell transplantation experiments indicate that aberrant IL-7R-mediated signaling increases the overall frequency of LPCs in Myc-induced leukemias. Taken together, these data highlight a synergy between mutant IL-7R and both Myc and Notch in inducing T-ALL in zebrafish and demonstrate that mutant IL7R enriches for leukemia-stem cell (LSC) potential in T-ALL.

No conflict of interest

D4. Hypoxia-inducible factor 2alpha is critical for NASH-related experimental liver carcinogenesis

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Introduction: Hypoxia and hypoxia inducible factors (HIFs) are believed to significantly affect the progression of chronic liver diseases (CLD). Recently, we showed that hepatocyte HIF-2alpha activation is a key feature in both human and experimental NAFLD and significantly contributes to disease progression. In the present study we investigated the contribution of hepatocyte HIF-2alpha in promoting the development of NAFLD/NASH-associated hepatocellular carcinoma (HCC). Materials and methods: The role of HIF-2alpha was investigated in human HCC liver specimens from NAFLD/NASH patients and in mice carrying hepatocyte-specific deletion of HIF-2alpha (HIF-2alpha fl/fl/Alb-Cre mice) receiving diethyl-nitrosamine (DEN) administration plus choline-deficient L-amino acid refined (CDAA) diet (DEN/CDAA protocol). Results and discussion: HIF-2 alpha, as detected by mRNA transcript and immunostaining, was expressed in HCC specimens from NAFLD/NASH patients, with higher expression in patients experiencing early tumour recurrence. Following the treatment with the DEN/CDAA protocol, mice carrying hepatocyte specific deletion of HIF-2 alpha showed a significant decrease in either the volume and the number of neoplastic liver tumour masses in transgenic mice as compared to control littermates. Liver tumours in HIF-2 alpha transgenic mice were also characterized by: i) a decrease of tumour associated macrophages and fibroblasts/myofibroblasts, as evaluated by F4/80 and alpha-smooth muscle actin immunohistochemistry, respectively; ii) a significant decrease in transcript levels for critical and HIF2alpha-related target genes, including c-Myc, CXCR4 and Cyclin D2.These results indicate that the activation of HIF-2alpha in hepatocytes has a critical role in the development of experimental liver carcinogenesis in a dietary NAFLD/NASH-related e environment.

No conflict of interest

Topic E
CLINICAL AND TRANSLATIONAL RESEARCH

E1.

Selected for oral presentation – Symposium I

E2. Tumor intracellular bioavailability of doxorubicin determines therapeutic efficacy of nanoparticle targeted to nucleolin

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Introduction: Liposomal doxorubicin (DXR) improved the safety profile over conventional free DXR, in patients [1]. However, those with breast or ovarian cancer did not benefit from improved efficacy relative to free DXR, contrasting with preclinical data. Hence, novel mechanisms of drug delivery are needed. It is thus hypothesized that targeting readily available overexpressed (and internalizing) markers, combined with efficient intracellular drug release, may offer increased efficacy and safety [2]. Herein, it was assessed in vivo, the mechanism of action of a novel DXR-containing pH-sensitive liposome functionalized with the nucleolin (NCL)-binding F3 peptide [3]. Materials and Methods: Tumor accumulation of liposomes containing DXR was assessed in female BALB/cnu/nu mice, bearing NCL-overexpressing tumors implanted in the mammary pad. A group of tumors was sectioned and blindly swept for image acquisition based on DXR fluorescence, using confocal microscopy. Antitumor effect against NCL-overexpressing tumors implanted in the mammary pad and orthotpic human mesothelioma tumors (epitheliod sub-type) was assessed upon i.v. administration of F3 peptide-targeted liposomes containing DXR (q7dx5w). Lip-DXR (5 mg/Kg), non-targeted non-pH-sensitive liposomal DXR, or saline were used as controls. Results and Discussion: Intracellular delivery of DXR at 7 mg/Kg by the liposomes functionalized with the NCL-binding F3 peptide, at the tumor level (assessed by the number of DXR+ cells per area), was 2.3-fold higher than the levels of (non-targeted) Lip-DXR, consistent with the enhanced intracellular delivery. This translated into a relevant improvement of overall survival (80% versus 43% for F3 peptide-targeted or non-targeted liposomes, respectively) in the NCL-overexpressing tumors implanted in the mammary fat pad, accompanied by a reduction of NCL-positive tumor blood vessels. Further proof of concept was extended to orthotopic (NCL-overexpressing) human mesothelioma, where liposomal DXR targeted to NCL enabled a 55-fold growth inhibition relative to the standard-of-care (combination of pemetrexed/cisplation). Overall, the antitumor efficacy of the NCL-based targeting strategy relies on NCL-dependent intracellular bioavailability of the delivered drug payload. Acknowledgments: FEDER funds through COMPETE, CENTRO 2020 and P2020, and ODD4PEGASEMP(reference 17646), QREN/FEDER MultiNanoMed(Ref: 23240) and ERDF/COMPETE 2020/FCT project POCI-01-0145-FEDER-007440.

I/we (In case of co-authors) have an interest in relation with one or more organisations that could be perceived as a possible conflict of interest in the context of the subject of this abstract. The relationship(s) is (are) summarised below: Moura, V. and Gregório, A. C. are employees of TREAT U, SA. All other authors declare no competing financial interests.

E3. Gene rearrangements in non-small cell lung cancer: towards the detection in cell free nucleic acids

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Introduction: The survival of lung cancer patients has greatly improved with target therapies. After a promising initial response, resistance is developed and patients undergo disease progression. Only a small part of the patients are eligible for target therapies. The assortment is performed through genetic analysis of the tumor and, based on the alterations, the patient is selected for the most suitable therapy. This analysis is usually performed on a tumor biopsy, or more recently, through liquid biopsy. For the patients harboring gene translocations the lack of liquid biopsy strategies is related to the difficulty of handling and evaluating cell free RNA (cfRNA). This work aims to make liquid biopsy accessible to lung cancer patients with gene rearrangements. By developing an approach that allows the simultaneous evaluation of DNA and RNA, our purpose is, not only to detect and monitor the driver alteration, but also the rise of resistance mutations. For that, we used plasma samples from stage IV lung cancer patients with EML4-ALK translocation at the time of progression. Cell free total nucleic acids (cfTNA) were extracted from the plasma and the cfRNA fraction was quantified by qPCR. Synthetic control samples were used to mimic different molecular status. Library preparation was performed using the Oncomine Lung Cell-Free Total Nucleic Acid Research Assay, that targets both mutations and gene fusion events. Samples were sequenced in the Ion S5XL sequencer and analyzed with Ion Reporter v5.6. We detected translocations, amplifications and mutations using reference control samples to validate the methodology. For the clinical samples, we observed that upon progression during Crizotinib treatment, the underlying resistance mechanism was the rise of an EGFR exon 19 deletion. This leads to the activation of the EGFR signaling pathway, regardless of the inhibition of ALK tyrosine kinase sustained by Crizotinib. These preliminary results provide evidence this methodology may hold potential application in monitoring EML4-ALK translocated tumors.

No conflict of interest

E4. Breaking the ctDNA sensitivity barrier

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Introduction: The study of tumor-derived DNA, as a liquid biopsy strategy, has revealed its clinical relevance as a biomarker for cancer management. However, the intrinsic low abundance of ctDNA and the complexity of the methodologies available difficult the detection of tumor mutations in plasma. Thus, we hypothesize that the use of cytotoxic chemotherapeutic drugs, as a driver of apoptosis, may increase the levels of ctDNA in circulation and enable the use of routine approaches for the detection of relevant mutations in plasma of cancer patients. In vitro, H1975 lung cancer cell line was treated with docetaxel for different periods of time (12h, 24h, 36h and 48h) to evaluate the time dependent effect of single dose treatment on the levels of apoptosis, using flow cytometry. The levels of ctDNA release were also assessed by quantification of DNA extracted from culture medium. In vivo studies were then performed in Rag2-/- IL2rg-/- immunodeficient C57BL/6 xenografted mice. The impact of docetaxel treatment on the levels of apoptosis in the tumor tissue was analyzed by immunohistochemistry, 24h and 48h after treatment. In parallel, cell-free DNA (cfDNA) was extracted from the plasma of xenografted mice to determine the effect of treatment on DNA release levels. The fraction of ctDNA within total cfDNA was determined by qPCR. The in vitro studies have shown an increase on the levels of apoptosis and ctDNA release upon docetaxel treatment, in a time dependent manner with greater impact at 48h. Similarly, the in vivo results have shown that a single dose treatment had an impact on tumor apoptosis, mainly at 48h, which correlated with increased levels of cfDNA detected in plasma. The specific detection of increased levels of tumor-derived DNA, by targeting a mutation of the xenografted cell

line, confirmed the influence of a single dose treatment on ctDNA release. This study provides new insights into a novel strategy which might overcome the low abundance of ctDNA and accelerate the standardization of liquid biopsy on the clinical routine.

No conflict of interest

E5. CDX2 targets, GPA33 and LI-cadherin, are novel biomarkers with prognostic value in gastric cancer

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Introduction: Gastric cancer is one of the most common types of cancer and the third cause of cancer-related death worldwide. The aggressiveness of the disease is related to, among other factors, tumour differentiation. CDX2 is a known intestinal differentiation marker with prognostic value in gastric cancer and two of its targets are expressed at the cell surface: GPA33 (glycoprotein A33) and LI-cadherin (liver intestine cadherin). Whether these membrane proteins are good biomarkers in gastric cancer and could refine the significance of CDX2 expression remains unknown. Materials and Methods: In order to answer these questions, we evaluated the expression of CDX2, GPA33 and LI-cadherin using immunohistochemistry in 350 gastric cancer samples arranged in TMAs (tissue microarrays) and evaluated the co-localization of the biomarkers using fluorescent multiplexed immunohistochemistry. Results and Discussion: CDX2 was expressed in 36% of the cases, while GPA33 and LI-cadherin were positive in 55% and 66%, respectively. All markers were significantly correlated with each other and are more often expressed in early stage (I and II) cancers ($p < 0.05$). Overall survival analysis showed that both GPA33 and LI-cadherin predict better outcome. When stratifying the series in early (I and II) and late (III and IV) stages, both proteins significantly associate with better outcome for late stages of disease progression. Overall, these data indicate that the presence of an intestinal differentiation programme in gastric cancer is a marker of good prognosis. The concordance rate between CDX2 and GPA33 and CDX2 and LI-cadherin expression was 68% and 64%, respectively. Since CDX2 displays a heterogeneous pattern of expression and TMA sampling can, by chance, select a negative area in a positive tumour, we also compared CDX2 expression between whole-tissue sections and TMAs. In our series we obtained 68 discrepant cases (positive for CDX2 in whole-tissue sections and negative in the TMAs). For the majority of these cases (50, corresponding to 74%) the combined expression of CDX2 targets, GPA33 and LI-cadherin, can rescue the underrepresentation of CDX2 expression in the TMAs and identifies CDX2-dependent intestinal differentiation. We conclude that CDX2, GPA33 and LI-cadherin are significantly associated with each other and are commonly expressed in early stages of gastric cancer. The expression of GPA33 and LI-cadherin is associated with better overall survival, particularly in late-stage disease patients. Finally, GPA33 and LI-cadherin are good cell surface surrogate markers for CDX2 expression, not only because of the good concordance of expression, but also because the combined expression of GPA33 and LI-cadherin rescues false negative CDX2 cases in TMAs versus whole-tissue samples.

No conflict of interest

E6. Normal minor allele frequency of the R450W RANK mutation in breast cancer patients: a large cohort retrospective study

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Introduction: The Receptor Activator of Nuclear Factor kB (RANK), is not only the major regulator of bone remodeling, but is implicated in bone metastasis and in breast cancer carcinogenesis and aggressiveness. We have previously identified in a Portuguese retrospective cohort a germline single nucleotide polymorphism (SNP), rs34945627, with an unexpected high minor allelic frequency (MAF; 12.5% (9/72) in BC patients, against 1.2% (1/80) in healthy controls; p=0.005). SNP rs34945627 leads to a C/T missense alteration (R450W). Therefore, we aimed to validate these findings in an independent clinical cohort and to characterize the nature of R450W mutation. Methods: For DNA genotyping we first optimized the utilization of rs34945627 TaqMan SNP Genotyping Assay to increase sensitivity and specificity, and introduced plasmid DNA as positive control for wild type and mutated SNP. We genotyped germline DNAs from 1,506 female breast cancer patients diagnosed between 1995 and 2011 and followed at the Oslo University Hospital. To characterize the nature of this genetic alteration, we performed functional studies using a homozygous model of mutant RANK overexpression in breast cancer and monocyte cell lines. Results and Discussion: Median age at diagnosis of patients in Oslo cohort was of 57.50 years (IQR:49.2-67.2), and median follow-up was four years (IQR:3-8). The majority of tumors were invasive (67.99%), of ductal histologic type (54.45%), and median tumor size was 18.0 mm (IQR: 12.0-25.0). The cohort includes 14.8% of tumors diagnosed in grade 1, 32.87% in grade 2, and 24.04% in grade 3. We successfully genotyped 1,426 samples, all wild-type (CC). Functional studies showed that R450W is a loss-of-function mutation which, in its homozygous form, decreases RANK pathway activity. Mutant breast cancer cells show decreased proliferation in vitro, and we also observed a decrease in tumor burden in an orthotopic xenograft mouse model of triple negative breast cancer. Moreover, mutant monocytes lose their ability to differentiate into osteoclasts, due to not sustained Nfatc1 expression. The use of a more sensitive genotyping method in a larger cohort revealed that SNP rs34945627 is present at identical MAF in breast cancer patients and normal population. Although this SNP lacks prognostic value in breast cancer patients, our findings revealed a new loss-of-function mutation in RANK, which can be further explored in the context of RANK pathway studies and inhibition.

No conflict of interest

E7. Gastric tumour dynamics and therapy response followed by a microRNA signature of circulating exosomes

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Introduction: There is no consensus regarding follow-up of Gastric Cancer (GC) patients. The available imaging tools and serological markers are poorly sensitive to monitor treatment response, minimal residual disease and tumour regrowth in a time-effective manner. We hypothesized that exosomes isolated from GC patients' plasma contain specific small RNAs, useful to monitor tumour dynamics, likely to change during the therapy, anticipating disease relapse. Material and Methods: We designed a prospective for the study of the small RNA profile of exosomes isolated from plasma of four GC patients collected at three timepoints: before, soon- and late-after surgery. Exosomes were isolated and characterized by ultracentrifugation and nanoparticle tracking analysis. Exosome and tumour RNA was extracted and profiled using small RNA sequencing technology (Ion Torrent). Bioinformatics analysis was performed using the tools bowtie2 and cufflinks for genome alignment, annotation and quantification. Analysis was focused on microRNAs (miRs), the most abundant class of small RNAs in these samples. Results and Discussion: On average, we detected 233 miRs in tumours and 175 in exosomes that were classified as present/absent and interrogated across samples. Cross-sectional analysis: same timepoint across patients. Longitudinal analysis: tumour and exosomes from the same patient. Cross-sectional analysis identified 49 miRs shared by all tumours, 33 by all exosomes collected before surgery, 83 by all exosomes collected after surgery. To pinpoint miRs useful to monitor tumour dynamics in each patient, two criteria were defined: 1) miRs present both in tumours and exosomes before surgery, and; 2) miRs present in tumours and absent in exosomes soon-after surgery. We found 133 miRs in patients A, 50 in B, 34 in C and 11 in D. Combining the cross-sectional and longitudinal analysis, we found two miRs fulfilling the above criteria

that were shared by three out of four patients. These two miRs were still detected in Patient D exosomes soon after surgery, likely reflecting non-curative surgery. Validation of these data is currently ongoing in a larger series of patients. Overall, this study pinpointed two miRs that may prove useful to monitor tumour dynamics and response to treatment in GC. The longitudinal analysis holds the promise of revealing a set of miRs with clinical utility for anticipating disease relapse, on a personalized manner.

No conflict of interest

E8. Patient-derived colorectal cancer explants: a tool for modelling cancer

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Introduction: More than 95% of anticancer compounds fail in clinical trials thus predictive models are still an unmet preclinical need. Tumor microenviroment contributes to this failure as it influences tumor progression, invasion and drug resistance. Moreover, cancer patient-derived models able to maintain the tumor microenviroment components, architecture and heterogeneity for a long-term period are missing. Our aim is to develop a model with patient-derived tumor explants (PDE) in dynamic culture systems to improve longevity and relevance of PDE. For this, we focused on colorectal cancer (CRC) as it is the third most common cancer worldwide and resistance to chemotherapy is well described. Materials and Methods: CRC samples were mechanically dissociated in approximately 1mm2 and PDE were cultured in agitation. Culture duration was determined by the frequency of sampling along culture time to assure a minimum of PDE concentration. Analyses regarding cell viability, proliferation and phenotype were performed by immunohistochemistry, resazurin reduction capacity and morphometric measurements, respectively. Results and Discussion: We successfully cultured 19 PDE with preservation of key pathological features. We observed maintenance of tumor epithelial cells for 1 month in all cases and up to 3 months in some cases. A decrease of the stromal compartment was also observed, with immune cells being progressively lost after two weeks. Optimization of the culture conditions is still ongoing, namely testing different media composition. Regarding DNA mismatch-repair proteins expression and microsatellite analysis, the original tumor status was preserved in all cases analysed. The same was observed for BRAFV600E and KRAS mutations with the exception of one case in which no KRAS mutation was detected in the primary tumour, but found in PDE since day 0. This can be due to initial sampling of two different tumour clones and has a translational relevance since it can impact treatment choice. Taken together, we were able to establish representative PDE dynamic cultures with preservation of general tumor architecture, heterogeneity and main cellular components. Furthermore, these PDE are amenable to potential drug predictive assays. Funding from Fundação para a Ciência e Tecnologia, Portugal (iNOVA4Health – UID/Multi/04462/2013 and PhD fellowship PD/BD/105768/2014 and PD/BD/128377/2017) is acknowledge.

No conflict of interest

E9. Loss of SOX9 expression is a predictive marker of relapse in gastric cancer

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Introduction: Gastric cancer is one of the most frequent tumors and the third leading cause of cancer-related death worldwide. The investigation of new biomarkers that can predict patient outcome more accurately and allow better treatment and follow-up decisions is of crucial importance. The transcription protein SRY-box 9 (SOX9) is a member of the high-mobility-group box class DNA-binding proteins. SOX9 is an important regulator

of cell-fate decisions in embryogenesis and adulthood, playing critical roles in differentiation and proliferation, also in the gastrointestinal tract. SOX9 has been correlated to tumor behavior in different tissues, including in gastric cancer, nevertheless with contradictory results. In this work we sought to ascertain the relevance of SOX9 transcription factor as a prognostic marker in gastric cancer. Material and methods: SOX9 expression was analysed by immunohistochemistry in a series of 333 cases of gastric adenocarcinoma, and its association with clinico-pathological and follow-up data was evaluated. A second gastric cancer validation cohort consisted of 354 cases from the cancer genome atlas (TCGA), showing high versus low SOX9 expression. Results and discussion: SOX9 expression was present in 83% of gastric cancer cases. Loss of SOX9 expression was significantly associated with relapse, however SOX9 expression was more frequent in stage IV cases. SOX9 loss of expression predicted worse disease-free survival but not overall survival, in this series. The prognostic value of SOX9 was independent of Laurén classification but it was more pronounced in tumors with expansive versus infiltrative growth (P=0.008). In patients that presented with disease in stage I to III, loss of SOX9 expression was significantly associated with venous invasion and lymph node metastases. In an independent series of gastric cancer, where SOX9 expression was assessed at the mRNA level (TCGA), low SOX9 expression levels were also significantly associated with poor patient outcome. Functional studies, after down- and up-regulation of SOX9, are now being undertaken in order to better understand the clinico-pathological observations and the relevance of SOX9 as a potential biomarker in gastric cancer.

No conflict of interest

E10. SOX2 predicts stage II CRC patients response to adjuvant chemotherapy

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Introduction: Colorectal cancer (CRC) remains a serious health concern, being the third most commonly diagnosed cancer in Europe and the fourth leading cause of cancer-related deaths worldwide. In Portugal, colorectal cancer is both the most frequently diagnosed and the one that causes more cancer-related deaths. The incidence of CRC increased in the last thirty years, strongly linked to changes in lifestyle and increased exposure to carcinogens, and it is expected to rise by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030. Despite advances in diagnosis and treatment, up to 30% of CRC patients subjected to tumour resection with curative intent, develop disease recurrence. The therapeutic approach after surgery is yet not consensual and depends to a large extent on disease stage. Anticancer drugs are among the most toxic agents administered to treat disease. Therefore, it would be desirable to have both prognostic markers to help stratify patients and thus help to identify who should be treated, and predictive biomarkers that foretold the likelihood of the benefit of administering a chemotherapeutic drug to cancer patients. With this project we intend to improve scientific insight into new molecular parameters that could help to distinguish whether specific subgroups of CRC stage II patients can effectively benefit from adjuvant therapy treatments. For this purpose, in our 236 stage II CRC patients cohort, we investigated, by immunohistochemistry, the biomarker potential of 3 transcription factors: CDX2, SOX2 and SOX9. We have also assessed V600E BRAF mutation status. In our CRC series, low CDX2 expression and de novo SOX2 expression significantly correlated with less tumour differentiation. SOX9, on the other hand, did not correlate with tumour grade. None of these molecules showed prognostic value for patients outcome in disease-free survival in CRC. However, in patients treated with adjuvant chemotherapy, de novo SOX2 expression significantly correlated with worse patient outcome ($p<0.01$). Accordingly, SOX2 has previously been reported to be expressed at higher levels in drug-resistant cells when compared to parental cells, in a colon cancer cell line. For further validation of SOX2 as a predictive biomarker of resistance to therapy, we are going to test the viability of CRC cell lines treated with 5-FU, after up- and down-regulation of SOX2 expression.

No conflict of interest

E11. Genetic counselling in hereditary diffuse gastric cancer: economical and psycho-social impact

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) syndrome is caused by CDH1-germline mutations and carriers have high-risk to develop early-onset diffuse gastric cancer (DGC) in both genders and lobular breast cancer (LBC) in females. HDGC is deadly for most of those expressing clinical phenotype. However, presymptomatic testing and identification of a predisposing mutation allows disease prevention or early-diagnosis in mutation-carriers through risk-reduction gastrectomy and yearly breast MRI, discharging all non-carriers. As Health-Care-Provider of the ERN-GENTURIS, we aim to evaluate the economic impact of genetic counselling, presymptomatic diagnosis and multidisciplinary care for the National Health Service (NHS), and the clinical and psychosocial implications for HDGC families. Material and Methods: We evaluated outputs of structured oncogenetics and high-risk consultations in 111 individuals at risk from 7 HDGC families by consulting clinical/financial records, and assessed its psychosocial impact by applying an emotional-distress-scale (HADS) to 70/111 individuals. Results and Discussion: From 2011-2016, we identified 7 apparently unrelated HDGC families carrying a deleterious germline CDH1 mutation (c.1901C>T; p.Ala634Val). From 111 individuals screened, 53% tested negative (n=58;30M:28F) and were discharged from preventive clinical follow-up, each costing 200€ to the Hospital. The remaining 47% (n=53;26M:27F) tested positive. From 7 probands, 2 were diagnosed with LBC and remain alive after curative surgery, while the 5 diagnosed with DGC are all deceased. The hospital expenses with probands range from 30-50K€ independently of cancer type. Costs with carriers opting for prophylactic approaches range from 4-8K€ (higher cost/females), except if disease is identified after the first high-risk consultation, raising the cost to 27K€, but life-saving. Those opting for surveillance, cost ~1K€/patient. Concerning psychologic impact, carriers demonstrate higher levels of distress than non-carriers. Therefore structured healthcare in HDGC economically benefits NHS, is life-saving for carriers, reassuring non-carriers.

No conflict of interest

E12. TP53 gene variants and associated phenotypes

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Introduction: Historically, breast cancer patients were only tested for TP53 germline mutations if they met criteria for LFS (Li-Fraumeni syndrome) or LFL (Li-Fraumeni like) syndrome, such as early-onset tumors including soft tissue sarcomas, breast cancers, central nervous system tumors, leukemias and adrenocortical carcinomas before the age of 45 years. With multigene panels being progressively implemented, unexpected TP53 families are being identified challenging our expertise for classification of gene variants and for clinical approach of these carriers. Objectives of this study are to characterize TP53 mutations identified and associated phenotypes, as well as to understand if a stratification of clinical management according to the type of mutation (dominant negative missense mutations, loss of function mutations) is possible. Materials and methods: 173 patients at-risk for HBOC, previously tested for BRCA1/2 mutations consented in genetic testing with a gene panel that included the analysis of TP53 point mutations by Next Generation Sequencing. Analysis of large rearrangements was performed by MLPA (Multiplex ligation-dependent probe amplification, MRC Holland). Mutation effect on the P53 protein was reviewed according to molecular databases, namely the International Agency for Research on Cancer TP53 Database (http://p53.iarc.fr). Clinical data from the new mutation carriers identified was reviewed and these results were integrated in a previous study that includes

LFS patients tested over the last 18 years. Results and discussion: Multigene testing found 3 additional families with germline TP53 pathogenic mutations. These families would not classify for traditional TP53 testing. After an overall review of TP53 mutation carriers, breast cancer and sarcomas were the most frequent cancers and missense, non-recurrent mutations were mostly observed. The majority of these missense mutations are located in the DNA binding domain of the protein responsible for the dominant-negative effect. Genotype-to-phenotype correlation is ongoing. Management of patients with LFS is complex and efforts are being made to differentiate the clinical management of LFS according to the type of TP53 variant. This stratification is particularly important since it may contribute to clarify the need for presymptomatic testing, not only in adults but also in children belonging to TP53 families.

No conflict of interest

Topic F
EXPERIMENTAL / MOLECULAR THERAPEUTICS, PHARMACOGENOMICS

F1. Targeting colorectal cancer proliferation and stemness using phytochemicals from broccoli and watercress in 3D cell models

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Introduction: Colorectal cancer (CRC) recurrence is often attributable to circulating tumor cells (CTCs) and/or cancer stem cells (CSCs) that resist to conventional therapies and foster tumor progression. Although, isothiocyanates (ITCs) derived from cruciferous vegetables of the family Brassicaceae have been demonstrated anticancer effects in CRC, little is known about their effect in CSCs and tumor onset. Materials and Methods: In this work, we examined the effect of ITCs-enriched Brassicaceae extracts derived from watercress and broccoli in cell proliferation, CSC phenotype and metastasis using a previously developed three-dimensional HT29 cell model with CSC-like traits – cell spheroids model. Both extracts were phytochemically characterized and their antiproliferative effect in HT29 cell monolayers was explored. Next, we performed cell proliferation assays and flow cytometry analysis in HT29 spheroids treated with watercress and broccoli extracts and respective main ITCs, phenethyl isothiocyanate (PEITC) and sulforaphane (SFN). Additionally, soft agar assays and relative quantitative expression analysis of stemness markers and Wnt/β-catenin signaling players were also performed to evaluate the effect of these phytochemicals in stemness and metastasis. Results and Discussion: Our results showed that both Brassicaceae extracts and ITCs exert antiproliferative effects in HT29 spheroids, arresting cell cycle at G2/M, possibly due to ITC-induced DNA damage. Colony formation and expression of LGR5 and CD133 cancer stemness markers were also significantly reduced. Only watercress extract and PEITC decreased the activity of the detoxifying enzyme ALDH1 in a dose-dependent manner, as well as β-catenin expression. Interestingly, watercress and broccoli extracts may target differently the Wnt signaling pathway by modulating the expression of different Wnt signaling players. Indeed, watercress extract may regulate Wnt signaling through the downregulation of β-catenin, whereas broccoli extract may modulate this signaling pathway by reducing TCF7L2 and AXIN2 expression hence, leading to downregulation of Wnt/TCF7L2 signaling. Hence, our research provides new insights and draws attention towards novel potential adjuvants on CRC therapy, the ITC-enriched Brassicaceae extracts, specially watercress extract, to target CSCs and CTCs by impairing cell proliferation, ALDH1-mediated chemo-resistance, anoikis evasion, self-renewal and metastatic potential.

No conflict of interest

F2. Precision therapy to distinct colorectal cancer subtypes to circunvent resistance to therapy and cancer stemness

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide due to metastatic disease and therapy resistance. Despite the efforts in chemotherapies, about 50-75% of patients can experience tumor recurrence. Recently, four consensus molecular subtypes (CMS1-4) have been proposed, associated to distinct prognosis and molecular signatures. Therefore, this raises the need to identify the best

treatment options to specific CRC subtypes. Materials and Methods: A panel of CRC cell lines was selected to represent different CRC subtypes: HCT116 [Microsatellite unstable (MSI), stage IV], LoVo (MSI, derived from metastases), SW480 [Microsatellite stable (MSS), chromosomal unstable (CIN)] and HT29 (MSS, CIN, BRAF mutated) and LS174T (MSI), the latter two representing mucinous tumors. Antiproliferative assays were performed by treating this cell line panel with cytostatic agents (5-Fluorouracil, Irinotecan and Oxaliplatin), nutraceuticals (Sulforaphane, derived from broccolis, and orange peel extract), histone deacetylase and methytransferase inhibitors (Vorinostat and Azacitidine) and signaling pathways modulators (e.g. Gant61), to determinate IC50 values and drug resistance. Cells were then treated with the IC50 of each compound and relative gene expression of markers of specific signaling pathways, stemness, epithelial-to-mesenchymal transition, proliferation and chemoresistance was performed by RT-qPCR. Results and Discussion: Our results showed that, overall, the SW480 cell line – MSS and resembling the CMS2 consensus molecular subtype, epithelial, CIN and with marked Wnt signaling activation - was the most sensitive to treatments. Contrary, HT29 and LS174T were the most resistant to conventional chemotherapeutic agents, namely to 5-FU, which is in accordance with the resistance of mucinous tumors to the standard of care CRC treatments. More importantly, the HT29, with the conjugation of BRAF mutation and MSS, molecular features associated to poor prognosis in CRC patients, was more resistant than LS174T. Interestingly, HT29 was the most sensitive cell line to Irinotecan, which together with the downregulation of stemness markers and Wnt signaling components, observed in response to this drug suggest that it may be promising to treat tumor subtypes resembling HT-29 features. In contrast to HT29, the metastatic LoVo and the mucinous LS174T, both MSI and expressing high CEA levels, showed resistance to Irinotecan. In summary, cell lines representative of distinct tumor subtypes have showed variable response profiles to different drug/compounds. Due to their transversal action, observed in all cell lines, in response to nutraceuticals, we hypothesize that these may help to circumvent drug resistance, possibly in synergy with specific chemotherapeutic regimens.

No conflict of interest

F3. Cytotoxic effect of pain management drugs in a metastatic prostate cancer cell line

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Introduction: Metastatic castration-resistant prostate cancer (mCRPC) patients inevitably succumb to this incurable disease. The chemotherapeutic agent Docetaxel (Doc) is the current first line treatment of mCRPC, increasing by 13.6 months the median overall survival of 14-20 months of patients. In this advanced state cancer-induced bone pain is the most prevalent burden in quality of life. Pain relief is achieved using local anesthetics (LA) as lidocaine (Lid), ropivacaine (Rop) and levobupivacaine (Lev) and morphine (Mor). Besides, a wide variety of cancer types reported cytotoxic, antiproliferative and sensitization to chemotherapy effects of LA. The aim of this project is to study the combined effect of pain management drugs with Doc in mCRPC treatment.Materials and methods: PC3 cell line from mCRPC was used to evaluate cytotoxic effects of LA and Doc in monotherapy. Cells were incubated with various concentrations of Mor (175.2 and 350.5nM), Lid 2% (426.7 and 853.4nM), Rop (36.4, 72.9, 136.7 and 273.3nM), Lev (43.3, 86.7 and 173.4nM) and Doc (0.01-500 nM) during 48 and 72 hours. Posteriorly cell proliferation was determined using sulforhodamine B (SRB) assay. Past 72h of incubation with LA in the previous concentrations the cells were treated with a concentration of Doc allowing 80% of maximal response (EC80) was added to unchanged medium with LA and fresh medium without LA. The effect of combined therapy was evaluated after 48 and 72 hours through SRB assay. Results and discussion: Results indicate a higher decrease in cell proliferation to 85.03%±2.75, 83.73%±2.17 and 85.43%±3.68, relatively to control, 48h following the administration of maximum concentrations of Lid, Rop and Lev respectively. The dose response study of PC3 cells to Doc treatment determined the EC80, 1.51 for 48h and 0.81nM for 72h. A decrease in cell proliferation was observed in the combined therapy of Doc with the higher concentrations of Lev to 72.71% and 53.10%. Preliminary data suggest that the administration of Doc in

the absence of LA substantially increases cell proliferation. Overall these results suggest that Lev is the most effective LA in sensitize PC3 cells to chemotherapy with Doc. The authors would like to thank Foundation for Science and Technology (FCT) (Strategic Project CNC.IBILI UID/NEU/04539/2013) through partnership arrangement PT2020 - COMPETE 2020 – Operational Thematic Program for Competitiveness and Internationalization (POCI) (POCI-01-0145-FEDER-007440) for financial support.

No conflict of interest

F4. Effect of plasma-activated medium and conditioned medium in retinoblastoma

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Introduction: Retinoblastoma (RB), a primitive neuroectodermal tumour, is the most common intraocular cancer in children. Recently, emerging reports on a new therapy based on plasma has shown a selective anticancer effect. Plasma is formed by ionization of gas atoms and molecules. Our previous results with direct cold atmospheric plasma (CAP) treatment revealed that CAP has an antiproliferative effect in RB cells. In order to explore CAP reactive potential, a new approach based on plasma-activated liquids has been studied. Recent evidences support that plasma-activated media (PAM) promote death in several cancer cell lines. Meanwhile, one of the problems with CAP is the difficulty of treating large solid tumours since plasma cannot fully penetrate the tumour. However, a possible paracrine effect between exposed and naïve tumoural cells may extend plasma effects beyond the undersurface of the tumour. Therefore, experiments with conditioned medium (CM) are essential to study this bystander effect. The aim of this work is to evaluate the effect of PAM and CM in human retinoblastoma cells and in fibroblasts. Materials and methods: PAM were prepared by exposing cell culture media to CAP up to 120 seconds. CM were prepared by exposing Y79 cell cultures to CAP for 120 seconds. Naïve Y79 and HFF1 cell cultures were incubated with PAM and CM as previously described for 24 hours. In order to assess the cytotoxicity of plasma in the RB cells and fibroblasts, Trypan Blue assay and SRB assay were performed. The production of intracellular peroxides, superoxide anion and anti-oxidative defences were also evaluated in RB cells. Results and discussion: PAM and CM leaded, respectively, to a cell dead of (35,82 ± 8,21)% and (37,39 ± 4,66)%, and a lower protein content (41,27 ± 26,06)% and (29,04 ± 10,52)% when compared to control. However, PAM and CM did not affect fibroblasts. Intracellular peroxide concentration decreased in (50,36 ± 12,98)% while superoxide anion concentration and antioxidant defences remained at normal levels with PAM. These results suggest that PAM has the potential to become a new adjuvant treatment for RB. Moreover, a paracrine effect between cells undergoing CAP treatment and naïve cells may be operating therefore expanding the range of plasma effects. Funding: FCT (Portugal) PEst-UID/NEU/04539/2013 and FEDER-COMPETE (FCOMP-01-0124-FEDER-028417 and POCI-01-0145-FEDER-007440).

No conflict of interest

F5. Targeting Breast Cancer with Cold Atmospheric Plasma

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Introduction: Breast cancer is the second most common cancer worldwide and the most frequent cancer among women with an estimated 1.67 million new cancer cases. The need for new, effective and free of side

effects therapies is growing as aging is modifying the epidemiology of cancer. Known as the fourth state of matter, cold atmospheric plasma (CAP) is a gas with enough energy to ionize a significant fraction of particles, has come into attention as a potential therapy of cancer. Previously, our studies showed that CAP determined the decrease of breast cancer cells viability after an exposure of only 60 seconds. The aim of this study was to evaluate the effect of CAP on breast cancer cell lines regarding reactive oxygen species (ROS), types of cell death and cell cycle. Materials and Methods: In this study, we used two different representative breast cancer cell lines: hormonal receptor positive breast cancer (MCF7) and triple negative breast cancer (HCC1806). Cells were cultured, plated and exposed to CAP, for different periods of time: 60 and 120 seconds and, using a homemade CAP ejector. To assess ROS intracellular concentration, cells were evaluated through specific probes, namely 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA) and dihydroetide (DHE). The levels of glutathione antioxidant defense (GSH) were also evaluated and studies were performed 2 and 24 hours after CAP exposure. Cell death type and cell cycle were assessed by flow cytometry using Annexin V/propidium iodide (PI) and PI, respectively. These studies were performed 24 hours after therapy. Results and Discussion: After 2 hours of CAP therapy, ROS levels do not show any variation compared to the control on MCF7 cell line. However, intracellular content of superoxide anion was (74.92±14.87) % on HCC1806 cell line exposed to CAP for 120 seconds. Levels of glutathione remained similar to the control. Our preliminary results suggest that apoptosis is the most prevalent type of death and treated cells are predominantly in G0/G1 phase on MCF7 cell cultures. Therefore, levels of oxidative stress and antioxidant defenses suggest that other events besides ROS formation might be involved in the plasma effect on breast cancer cells. These results encourage further studies to understand the possible mechanisms of action. Funding: FCT, Portugal (UID/NEU/04539/2013), FEDER-COMPETE (FCOMP-01-0124-FEDER-028417, POCI-01-0145-FEDER-007440).

No conflict of interest

F6. In vitro evaluation of cytotoxic effect of cisplatin, docetaxel and 5-fluorouracil in FaDu cell line - preliminary results

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Introduction: Head and neck cancer include tumors occurring in upper aerodigestive tract. One of the most common type of head and neck squamous cell carcinoma (HNSCC) is hypopharyngeal cancer. Poor prognosis and lower survival are characteristics of HNSCC. Cisplatin (CDDP) and docetaxel (DTX) are the chemotherapeutic agents used in HNSCC patients. In this study we evaluated cytotoxic effects of CDDP and DTX in FaDu cell line. Materials and Methods: To evaluate proliferation, FaDu cell line was incubated with increasing concentrations of CDDP (0.5-33 µM) and DTX (0.01-180 nM) for 24, 48 and 72h, and sulphorhodamine B assay was performed to determine inhibitory concentrations (IC50, IC20 and IC80). Cell cycle (propidium iodide), death (Anexin V and propidium iodide), superoxide radical (dihydroethidium), peroxides (2,7-dichlorodihydrofluorescein) and intracellular levels of reduced glutathione (mercury orange) were evaluated by flow cytometry after 48h with IC20; 50; 80 of CDDP and DTX. Results and discussion: IC20, IC50, IC80 values of CDDP and DTX at 48h were 6.2, 12.3, 24.4 µM and 1.35, 5.57, 23.01 nM, respectively. After 72h, IC20, IC50, IC80 values were 5.92, 10.20, 17.54 µM for CDDP and 1.21, 2.65, 5.83 nM for DTX, respectively. CDDP induced a decrease of cells viability statistically significant for IC50 ($p<0.01$) and IC80 ($p<0.001$), translated in increase of cell death by necrosis and apoptosis for IC50 ($p<0.05$;$p<0.001$) and IC80 ($p<0.001$) respectively. The IC20 of CDDP induced a cell cycle arrest in G2/M ($p<0.01$). Higher concentrations induced a blockade in S phase ($p<0.01$ for IC50 and $p=0.001$ for IC80), along with an increase of intracellular levels of radical superoxide ($p=0.03$) and GSH ($p=0.05$). Moreover, an increase of intracellular levels of with IC20 was observed ($p<0.01$).

DTX had significant effect in reduction of viability for all doses (p<0.001), along with an increase of apoptosis (p<0.01 for IC20, IC50 and p=0.001 for IC80) and necrosis (p=0.001 for IC50, p<0.001 for IC80). GSH levels increased with IC80 (p<0.001) and peroxides levels decreased with DTX IC50 (p<0.05) and IC80 (p<0.001). Preliminary results showed cell arrest in G2/M with IC80 incubation. Adding, radical superoxide levels increased with DTX IC80. All statistical differences mentioned are relative to control. CDDP and DTX cytotoxic effects in FaDu cell line were time, dose and ROS-dependent.

No conflict of interest

F7. Development of an anti-cancer cell therapy with genetically-modified mesenchymal stem/stromal cells expressing azurin

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Introduction: The application of anti-cancer therapeutic agents is often constrained by their short half-life or generalized toxicity. Recently, cell-based therapies have been explored as a promising alternative to enhance their effects by improving specificity and robustness. Human mesenchymal stem/ stromal cells (hMSCs) hold a promising future for cell-based therapies due to their unique biological features. Taking advantage of their innate tropism for tumors, genetically engineered versions of MSCs have been under development as cell delivery systems for several anti-cancer proteins. In this study, we engineered hMSCs to secrete a human codon-optimized version of azurin (hazu), a bacterial protein that in previous studies demonstrated anti-cancer activity towards different cancer models. Methods: hMSCs derived from bone marrow (BM) and umbilical cord matrix (UCM) were engineered through non-viral transduction methods. In a first approach, cells were microporated with a pVAX-azurin plasmid (hazu-MSCs), leading to a transient gene expression of azurin and its secretion to the culture media (referred to as conditioned medium (CM)). Cancer cell proliferation was assessed upon exposition to hazu-MSCs CM by MTT assay. Aiming at the establishment of a stable azurin-expressing MSC cell line, we developed a CRISPR/Cas9 strategy to guide the insertion of the hazu gene targeting the first intron of PPP1R12C gene, recognized as a genomic safe harbour (GSH). The hazu gene will be under the control of the IL6 gene promotor sequence in the repair template, envisaging a controlled expression of the knocked-in gene within the tumor microenvironment. Results and Discussion: Azurin was detected in the conditioned media of transfected BM- and UCM-MSCs, confirming the ability of MSCs to express a human codon-optimized DNA sequence of azurin. We observed a decrease in cancer cell proliferation upon treatment with hazu-MSCs conditioned media in a 2D monolayer configuration for breast (MCF-7) and lung (A549) cancer cell lines. Moreover, MSCs' migratory tropism towards colon (HT-29) and breast (MCF-7) cancer cell lines was confirmed in 3 different BM-derived MSC donors, through indirect co-cultures under physiological barriers such as Collagen type I and Matrigel. Regarding the genome editing strategy, a guide RNA sequence was obtained, targeting the correct intron in HEK293T cells. Most of the fragments comprising the repair template were obtained, and thus the next step will be the production of a repair plasmid able to repair the DSB and insert our engineered construct in the correct location.

No conflict of interest

F8. PDT based on novel 4,5,6,7 - tetrahydropyrazolo [1,5-a] pyridine fused chlorins against melanoma, bladder, and oesophagus cancer cells

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Introduction: Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic procedure, which is entering the mainstream of cancer treatments. Nowadays PDT has been successfully used in the treatment of several types of cancer which can be easily exposed to light due to a superficial location or by endoscopy. Material and Methods: The human melanoma cells A375, the human bladder cancer cells HT1376, and the human oesophagus cancer cells OE19 were submitted to a series of new the photosensitizers 24 hours prior irradiation with a proper device. The formulation of the sensitizers consisted in a 1 mg/mL solution in DMSO and the desired concentrations (from 1 nM to 10 mM) being achieved by successive dilutions. Controls were performed on every test. Cells were washed with PBS and new drug-free medium was added immediately before the irradiation. Each plate was irradiated with a fluence rate of 7.5 mW/cm2 until reaching 10 J. Evaluation by MTT assay was performed 24 h after the photodynamic treatment. Results and Discussion: Our previous in vitro PDT studies demonstrated that increasing the hydrophilicity of the chlorins leads to higher activity against A375 melanoma cells. Therefore, a series of novel 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused chlorins bearing dicarboxylic acid and monocarboxylic moieties were developed showing an interesting biological activity against A375, HT1376 and OE19 cancer cells. Inhibition of the metabolic activity seems to be dependent on the concentration of the sensitizers used. With the experimental metabolic activity values, it was possible to calculate the concentration of the sensitizers that inhibits the proliferation of cultures in 50% (IC50). For this series of compounds, IC50 values ranged from □M to nM concentrations. Nevertheless, a new molecule with an IC50 value of 67,93 nM stood out. This interestingly low IC50 values in the nanomolar range encourage further studies. This work was supported by the Foundation for Science and Technology: POCI-01-0145-FEDER-PTDC/QEQ-MED/0262/2014 (COMPETE 2020), POCI-01-0145-FEDER-007630 and POCI-01-0145-FEDER-007440.

No conflict of interest

F9. Human amniotic membrane extract as an approach to hepatocarcinoma: effects of protein fractionation and thermal denaturation

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Introduction: Hepatocellular carcinoma (HCC) is the most common liver cancer, being one of the most incident and of the deadliest worldwide. HCC is mostly diagnosed at late stages which leads to a poor diagnosis and treatment efficiency. Human amniotic membrane (hAM) presents immunoregulatory, anti-angiogenic and pro-apoptotic activity. Previous work of our team showed that total human amniotic membrane extract (hAME) leads to decreased viability and increased cell death on HCC cells. Thus, with this work we aimed to evaluated the effect of different hAME fractions on HCC cells. Methods: Having previously verified that hAME comprises a complex protein mixture, fractionation was performed considering solubility, through ammonium sulphate (AS) precipitation. Sequential precipitation was performed by adding 10%, 25% and 50% AS to hAME, under continuous incubation on ice for 15min, followed by centrifugation at 14000 G, 15 min. Precipitated fractions (10P, 25P and 50P) were resuspended on PBS and soluble fractions (10S, 25S and 50S) were submitted to salting out with PBS by centrifugation at 4000G, during 60 min on centrifuge tubes with a membrane pore with a 30 kDa cutoff. hAME was also submitted to thermal denaturation at 100ºC, during 5min at a dry bath. HCC cell lines HepG2, Hep3B and HuH7 were incubated with a protein concentration of 1µg/µL, during 72h. Metabolic activity was accessed by MTT assay. Results and Discussion: Our results demonstrated that incubation of HCC cells with hAME induced a decrease on metabolic activity, compared to control (HepG2: 57.07±9.26; Hep3B: 50.66±9.78; HuH7.sil: 58.16±6.05). Incubation with fractions obtained from AS precipitation induced a lower metabolic activity compared to incubation with total hAME. Values of metabolic activity varied from 2 to 30%, depending on HCC cell line, which indicates that fractionation could be isolating

hAME components that could be responsible for hAME effects on HCC. Thermal denaturation of hAME did not prevent metabolic activity as effectively as hAME (HepG2: 71.79±9.65; Hep3B: 87.59±6.33; HuH7: 84.17±5.70). These findings indicate that proteins are, at least in part, the components responsible for the main anti-tumor activity of hAME. Funding: FCT Portugal (SFRH/BD/116794/2016); PEst-UID/NEU/04539/2013 and FEDER-COMPETE (FCOMP-01-0124-FEDER-028417 and POCI-01-0145-FEDER-007440), FIS-INFARMED (FIS-2015-01-Onc-20150630-120).

No conflict of interest

F10. IS COLD ATMOSPHERIC PLASMA CAPABLE OF KILLING BLADDER CANCER CELLS?

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Introduction: Bladder cancer (BC) is a solid tumor with high recurrence rates. It is the sixth tumor with the highest incidence and the eighth one with the highest mortality in the world. Plasma is one of the physical states of matter, in which a certain portion of the particles is ionized. Our aim was to evaluate the cytotoxicity and the oxidative stress of cold atmospheric plasma (CAP) in a human bladder cancer cell lines. Materials and Methods: An electronic device able to generate high output voltage was designed to create plasma with the aim to study its effect in human cells. Two BC cell lines, HT1376 (grade 3, carcinoma) and TCCSUP (grade IV, transitional cell carcinoma), were cultured. Cells were seeded in 48 and 24-multiwell plates, at a density of 0.1x106 and 0.3x106 cells/mL, respectively, and left overnight. Then both cell lines were submitted to CAP treatment. CAP was generated in open air, 2 mm above the surface of the cell cultures medium, during short periods of time (15s, 30s, 60s, 90s and 120s). Protein content was assessed by SRB assay and cytotoxicity by MTT assay, performed 24 hours after treatment. Also, oxidative stress and antioxidant defenses studies were performed 2, 6 and 24 hours after treatment. Results and Discussion: After CAP treatment, protein content of both cell lines decreased accordingly to the exposure time. By MTT assay, we observed a decreased metabolic activity, also for both cell lines. These results show that CAP treatment was able to induce a significant reduction of total protein content and metabolic activity even after short periods of exposure. In terms of oxidative stress, for TCCSUP cell line, results demonstrate a tendency to increased intracellular production of radical superoxide and a tendency to decreased intracellular production of peroxides. We observed similar tendencies for HT1376 cell line. Nevertheless, we could not observe a significant difference in expression of GSH levels for TCCSUP and HT1376. CAP can potentially offer a minimally-invasive option that allows specific cell removal without interfering with the whole tissue.

No conflict of interest

F11. Agregation in Biological Medium of Promising Theranostic Agents for Cancer: An unwanted Reality

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Introduction: We recently developed a new type of photochemically stable platinum (II) chlorins, which are remarkable photosensitizers that can be used in photodynamic therapy (PDT), due to its therapeutic capacity. Simultaneously, due to its highly luminescence proprieties, in the biological relevant 650-850 nm red and near infrared spectral region, they may be used for biological imaging. In addition, photophysical studies indicate that they may be used as ratiometric oxygen sensors. Materials and methods: Compounds with different degrees of hydrophilicity were synthesized and characterized photochemically and photophysically.

Photocytotoxicity studies were carried out against two human tumor cell lines, the OE19 line of oesophageal carcinoma and the A375 line of melanocytic melanoma, in order to test their potential therapeutic effect in PDT. For this, cells were plated and kept in the incubator overnight. Each sensitizer was solubilized in dimethyl sulfoxide and administered in several concentrations. Cells were incubated for 24 h. After that, plates were irradiated with a fluence rate of 7.5 mW/cm2, to reach 10 J. Evaluation by MTT assay was performed 24 h after the photodynamic treatment. With the experimental values was calculated the IC50. Results and discussion: Preliminary cytotoxicity studies indicate that, in both cell lines, platinum (II) chlorins with more hydrophilic features require lower doses of photosensitizer to induce a significant photocytotoxic effect on tumour cells. The most interesting results were IC50 values of 165.9 nM (confidence interval at 95 %: [77.9; 356.6]) for A375 line and 498.6 nM (confidence interval at 95 %: [283.5; 876.5]) for OE19 line. However, it was hypothesized that this type of compounds might be aggregating in the biological medium, therefore, decreasing its photodynamic capacity. This hypothesis was corroborated by additional photophysical studies. As such, disaggregation was performed with the use of surface active agents (surfactants). In fact, surfactants have been widely used for enhancing solubilisation of poorly soluble drugs. Although photocytotoxicity studies reveal that the platinum (II) chlorins tested would be very promising for PDT, further cytotoxicity studies will be carried out using a novel formulation. This work was supported by the Foundation for Science and Technology: POCI-01-0145-FEDER-PTDC/QEQ-MED/0262/2014 (COMPETE 2020); POCI-01-0145-FEDER-007630 and POCI-01-0145-FEDER-007440.

No conflict of interest

F12. A novel steroidal B-ring modified aromatase inhibitor: comparative study with exemestane in estrogen-dependent breast cancer cell

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Introduction: Aromatase inhibitors (AIs) are the first-line therapy for Estrogen-receptor positive (ER+) breast cancers. Although, despite their therapeutic success, AIs-acquired resistance may occur, which justifies the search for new and potent AIs. Based on the interaction of the substrate androstenedione with aromatase, in the last years our group have studied different modified A-/B-ring steroidal AIs [1]. In addition, we have shown the biological mechanisms of Exemestane (Exe), a steroidal third-generation AI, in breast cancer cells [2]. In this study, we compare the anti-aromatase and biological activity of compound 68, a B-ring modified steroidal AI with Exe. Materials and methods: The effects of 68 in enzymatic activity/stability of aromatase were studied in an aromatase-overexpressing ER+ breast cancer cell line, MCF-7aro, through a radiometric assay and western blot. The anti-proliferative effects were studied through MTT assays and flow cytometry. The caspase-7 activity was evaluated by a chemiluminescent assay and the production of ROS and the mitochondrial transmembrane potential (ΔΨm) were explored by fluorescence assays. Cell morphology was analysed by Giemsa/Hoescht staining. Moreover, it was studied the anti-proliferative efficacy of this compound in a resistant breast cancer cell line that mimics late-resistance to Exe, LTEDaro. Results and discussion: Like Exe, compound 68 presents anti-aromatase activity and has the ability to reduce aromatase expression in MCF-7aro cells. Steroid 68 induces a decrease in MCF-7aro cell viability and cell cycle arrest at G0/G1 similar to Exe. Moreover, compound 68, as well as Exe, increases ROS production, decreases ΔΨm and cause typical morphological features of apoptosis. Preliminary results suggest that it also increases caspase-7 activity. Curiously, AI 68 is capable of sensitise the Exe-resistant cells as shown by the decrease in LTEDaro cell viability. Thus, these results show that steroid 68 may be a promising AI. In addition, this work provides new insights on the steroidal core structure in order to design new and more potent AIs. Acknowledgements: FCT for C. Amaral (SFRH/BPD/98304/2013) and T. Augusto (BD/128333/2017) grants and for financial support (UID/MULTI/04378/2013–POCI/01/0145/FEDER/007728); Shiuan Chen (Beckman Research Institute, USA) for MCF-7aro/LTEDaro cells. References: [1] Varela CL. et al. (2016) Bioorg Med Chem;24(12):2823-31. [2] Amaral C. et al. (2012) PLoS One;7(8): e42398.*No conflict of interest*

F13. Cytotoxicity of Novel Pyridine-fused Chlorins as promising PDT agents against oesophagus cancer cell line

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Introduction: The high selectivity of photosensitizers to solid tumors and the generation of cytotoxic reactive oxygen species (ROS) near the target minimize the side effects usually observed with common systemic drugs, giving photodynamic therapy (PDT) several advantages over the classic anticancer therapies. Here we describe the development of new pyridine-fused chlorins that not only show enhanced chemical and structure stability by the introduction of a fused ring, but also increased hydrophilicity. These characteristics in association with a rich pattern of absorption bands within the phototherapeutic window make these compounds very active photodynamic agents. The aim of this work is to disclose the synthetic details, structural characterization and cytotoxicity evaluation of these very promising PDT agents against oesophagus cancer cell line (OE19). Material and Methods: Novel 4,5,6,7-Tetrahydropyrazolo[1,5-a]pyridine–fused chlorins were synthesized via [8π + 2π] cycloaddition of diazafulvenium methides with tetrakis(pentafluorophenyl)porphyrins. These chlorins underwent nucleophilic aromatic substitution to afford the corresponding PEGylated derivatives. The colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was used to evaluate the effect of these compounds on the metabolic activity. For this study, previously cultured OE19 cells were seeded in 48-well plates and, after 24h, incubated with increasing concentrations of these compounds (50nM – 10uM). After 24h, the cells were irradiated with a proper light source (λcut-off <560nm) until a total of 10 J. MTT assay was performed 24h later. Dose response curves were plotted and IC50 (half-maximal inhibitory concentration) values for each compound were determined. Results and Discussion: Although the di-ester chlorin showed IC50 values between 5 uM and 10 uM, their corresponding diol and PEGylated derivatives showing higher cytotoxicity with IC50 values up to only 250 nM,. All of the studied compounds revealed dose-dependent anti-proliferative effects, and derivatives with increased hydrophilicity obtained by either the reduction of di-ester to diol derivative or by the addition of PEG moieties, showed a much higher activity against the OE19 cancer cell line. This work was funded by Fundação para a Ciência e a Tecnologia (FCT): POCI-01-0145-FEDER-PTDC/QEQ-MED/0262/2014 (COMPETE 2020); POCI-01-0145-FEDER-007630 and POCI-01-0145-FEDER-007440.

No conflict of interest

F14. Modulation of membrane order and interaction with lipid rafts by azurin increases sensitivity to anti-cancer drugs

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Introduction: Membrane lipid rafts are highly ordered microdomains and essential components of plasma membranes. In particular, caveolae, a subset of lipid rafts characterized by the presence of caveolin-1, are gaining increasing recognition as mediators in tumor progression and resistance to standard therapies [1,2]. Material and Methods: A combination of biochemical and biophysical approaches were used to evaluate the effects of azurin at the lipid raft organization. The broad effect of azurin at the cell surface level was examined by Atomic Force Microscopy and plasma membrane order was assessed by two-photon microscopy usinf the

Laurdan probe. Results and Discussion: We demonstrate that azurin uptake by cancer cells is, in part, mediated by GM-1 and caveolin-1, lipid rafts' markers. This recognition is mediated by a surface exposed hydrophobic core displayed by azurin since the substitution of a phenyalalanine residue in position 114 facing the hydrophobic cavity by alanine impacts such interactions, debilitating the uptake of azurin by cancer cells. WT azurin inhibits growth of cancer cells expressing caveolin-1, which is only partially observed with mutant azurin. The simultaneous administration of azurin with anticancer therapeutic drugs (paclitaxel and doxorubicin) results in an enhancement in their activity, contrary to the mutated protein. In particular, in lung cancer, the Epidermal Growth Factor Receptor (EGFR) is one of the main targets for clinical management of this disease. The effectiveness of therapies towards this receptor has already been linked to the expression of integrin receptor subunit β1 in NSCLC A549 cells, with descriptions of being located in lipid rafts. We also demonstrate that azurin controls the levels of integrin β1 and its appropriate membrane localization, impairing the intracellular signaling cascades downstream these receptors and the invasiveness of cells. We show evidences that azurin when combined with gefitinib and erlotinib, tyrosine kinase inhibitors which targets specifically the EGFR, enhances the sensitivity of these lung cancer cells to these molecules [3]. Using AFM to determine The Young 's module (E), we showed that the stiffness of A549 lung cancer cells decreased with exposure to azurin and also gefitinib, suggesting that the alterations in the membrane properties may be the basis of the broad anticancer activity of this protein [3]. In addition, treating of cancer cells with azurin alters the lipid raft exposure at plasma membrane and causes a decrease in the plasma membrane order. Overall, these results show that azurin may be relevant as an adjuvant to improve the effects of other anticancer agents already in clinical use, to which patients often develop resistance hampering its full therapeutic response.

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No conflict of interest

F15. Developing exosome-based drug delivery systems for targeting breast cancer brain metastases

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Introduction: The treatment of metastatic breast cancer (MBC) is challenging. As new and effective therapeutic approaches allow patients to survive longer to a breast primary tumor, a metastatic disease often develops. Effective and selective drug delivery systems (DDS) reaching these metastases are valuable clinical tools for fighting MBC. A major obstacle for an effective drug delivery is the physiological mismatch observed between the cell membrane and the membrane of the typical liposomal carriers used as DDS. Exosomes are natural cell-derived vesicles that perform bio-functions with high targeting specificity. Exosomes present a lipid bilayer enclosing a small fraction of the cytosol and transport a cargo of proteins. When compared to other nanoparticle delivery vehicles, exosomes decrease the development of immune responses and cross biological barriers offering an alternative approach to conventional DDS. In this work, exosomes from breast cells were isolated aiming for their characterization for potential DDS applications. We also focused in the interaction exosome-cell penetrating peptides (CPPs) envisioning the development of an exosomal-based DDS capable of reaching breast cancer brain metastases. The combination of potential anticancer peptides (ACPs) with human-derived exosomes might confer increased homing ability, efficacy and selectivity to the DDS. Materials and Methods: The CPP activity on human breast cells was followed by viability and atomic force microscopy (AFM) studies. Exosomes were isolated using a commercially available kit and characterized using biophysical and imaging techniques (surface charge, transmission electron microscopy (TEM), AFM). Results and Discussion: Results suggests an intracellular target for the CPP cytotoxic activity on breast cells. The binding of the peptide to the membrane of human cells and exosomes results in changes of its surface charge and membrane dipole potential that can be correlated with the peptide's activity. Acknowledgements: Fundação para a Ciência e a Tecnologia (FCTI.P., Portugal) is acknowledged for funding—PTDC/BBBBQB/1693/2014. F. D. O., T. N. F. and D. G. acknowledge FCT I.P. for fellowships PD/BD/135046/2017, SFRH/BD/5283/2013 and SFRH/BPD/

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No conflict of interest

F16. The effect of a sod mimic (MnTnHex-2-PyP) on the viability and migration of human renal cancer cells

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Introduction: Manganese(III) porphyrins (MnPs) mimic the natural superoxide dismutase enzymes (SOD) and modulate the cellular redox status by scavenging a plethora of different reactive oxygen species (ROS) and by the redox regulation of signaling pathways. MnPs have been developed as potential drugs for different pathologies and are also useful mechanistic tools to assess the involvement of oxidative stress in pathological and toxicological conditions. MnPs are also promising drug candidates for redox-based therapeutic approaches against cancer. Such use is currently being evaluated in different clinical trials. The present work constitutes a first attempt to explore the potential of the SOD mimic MnTnHex-2-PyP as a potential drug against renal cancer. Material and Methods: In this study, the human renal cancer cells (786-O) were treated with increasing concentrations of MnP (0.1-25 µM) for different exposure times (12-48 h). The Crystal Violet and MTS assays were used to evaluate the cell viability. The impact of MnP in 786-O cell cycle and cell death was investigated by assessing the cellular DNA content using PI stain in fixed cells. Intracellular ROS were analyzed by flow cytometry using the fluorescence probe dihydrorhodamine 123. The impact of MnP (0.25 µM) in 786-O collective cell migration was evaluated by the wound-healing assay. The effect of this compound in chemotaxis was assessed by the transwell assay using FBS as the chemoattractant. Results and Discussion: MnP exposure resulted in a concentration and time-dependent decrease in cell viability. The exposure to MnP (5 µM) led to a significant increase in sub-G1 population. Moreover, the exposure to MnP resulted in a concentration-dependent increase in intracellular ROS, presumably due to the generation of H2O2 during the dismutation of O2.-. The MnP did not lead to a reduction in collective cell motility. Nevertheless, the MnP significantly decreased the chemotactic migration of human renal cancer cells.Overall, these results suggest that MnTnHex-2-PyP may have a beneficial impact in reducing renal cancer cells viability and migration and warrant further studies regarding SODm-based therapeutic strategies against human renal cancer.

No conflict of interest

F17. Antibody based therapy for T-cell Acute Lymphoblastic Leukemia through Targeting IL-7 receptor

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Introduction: IL-7 and IL-7Rα have been implicated in T-cell acute lymphoblastic leukemia (T-ALL) development. In more than 70% of T-ALL cases, IL-7 induces proliferation of leukemia cells, and 10% of T-ALL patients display IL-7Rα gain-of-function oncogenic mutations. Thus, targeting IL-7/IL-7Rα may be a valid strategy for the

treatment of T-ALL. Fully human monoclonal antibodies are particularly useful for pharmaceutical applications as these products display reduced immunogenicity. Here, we describe the isolation of a fully human antibody against IL-7Rα using phage display, which we thoroughly characterize. Materials and Methods: Phage display was applied to generate human antibodies against the extracellular domain of IL-7Rα. Selected antibody, named B12, was engineered into full length IgG1 format in CHO cells. Antigen recognition was determined on Ba/F3 cells ectopically expressing the receptor, and various T-ALL cell lines and patient samples. IL-7R signaling was evaluated by western blot. Rate of B12 internalization and intracellular trafficking was elucidated by confocal microscopy. In vivo effect was tested in Rag-/- mice transplanted with D1 cells expressing mutant IL-7Rα. Site specific modification of B12 generated an antibody-drug conjugate (ADC) with monomethyl auristatin E (MMAE). Results and Discussion: Using combinatorial phage display libraries, we isolated and characterized a fully human monoclonal antibody against IL-7Rα. The antibody, expressed in IgG1 format, recognizes specifically human IL-7Rα in different cell types, and inhibits IL-7R-dependent signaling. Importantly, B12 delays tumor progression in a systemic mouse model of mutant IL-7Rα gain-of-function T-cell leukemia, with a major impact on leukemia cell dissemination in different organs. Because our antibody against IL-7Rα is internalized via clathrin-coated pits and traffics to early endosomes and then to lysosomes, we expand its potential for targeted therapy through the generation of an MMAE ADC that shows improved target cell killing abilities in vitro. Our studies serve as a stepping stone towards the development of novel targeted therapeutic strategies in T-ALL and other malignancies and pathologies where IL-7 and IL-7Rα may be involved.

No conflict of interest

F18. Unraveling the molecular targets of new ruthenium compounds towards colorectal cancer therapy

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Introduction: Colorectal cancer (CRC) is an important cause of global morbidity and mortality. During colorectal carcinogenesis, the cells acquire several mutations that are crucial for CRC development. The most frequent mutations found in this type of cancer are KRAS, BRAF and PIK3CA. Nowadays, there are limited chemotherapeutic agents available for the treatment of CRC, which is frequently accompanied by severe side effects and acquisition of resistance. Moreover, CRC that harbor mutations in KRAS, BRAF and PIK3CA associated with EGFR overexpression do not respond to EGFR inhibitors available. This constitutes a clinical relevant problem that needs to be overcome. Ruthenium (Ru) drugs had arisen as one of the most promising metallodrugs with features that increase their specificity and selectivity toward cancer cells. For these reasons, three new multifunctional polymer-metal conjugates of ruthenium (RuPMC) were synthesized, one taking advantage of Ru anticancer properties (PMC79) and two resulting from Ru functionalization to improve the targeting approach (PMC78 and PMC85). In this work, we wanted to understand if these new Ru compounds are promising candidates for CRC therapy. For that, we used two CRC-derived cell lines with different genetic background and studied the effect of Ru compounds on cell proliferation, cell death, MAPK-ERK and PI3K-AKT signaling pathways, actin cytoskeleton and GLUT1 expression. The results showed that our compounds induce apoptosis but do not interfere with cell cycle. Moreover, the Ru compounds seem to influence differently the expression of AKT and ERK in the two CRC cell lines. PMC79 inhibited to a high extent the expression of AKT and ERK proteins in CRC cells with KRAS mutation. RuPMCs also affected F-actin polymerization and β-actin expression suggesting that actin might be a possible target for these compounds. Additionally, PMC79 upregulated the expression of GLUT1 in CRC cells with KRAS mutation, and the combination of PMC79 with a GLUT1 inhibitor potentiated the Ru compound effect. Overall our results showed that all compounds present promising anticancer activity in CRC cells. Our data suggest that RuPMCs have a more pronounced effect on CRC harboring KRAS mutation, what could bring new avenues in CRC therapy.

No conflict of interest

F19. Elucidating antibody diffusion in the tumor microenvironment using 3D cell models

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Introduction: In recent years, numerous anti-cancer antibodies (Ab) have been approved and applied in the clinics, contributing to the emergence of cancer targeted therapies. Despite their success, Ab therapies present limitations which may hamper their effect and allow tumor progression. Ab efficacy and efficiency are necessarily dependent on the reaching the target cells within the tumor environment (TME). The latter is composed by ECM, soluble molecules and distinct cell types, all of which may interact with the Ab and ultimately affect its transport. This in turn depends on factors associated with the characteristics of the Ab such as its size, surface charge and structure and also factors dependent on the TME, such as pH and ECM composition. Here, we propose to study Ab diffusion in the heterogeneous tumor tissue. For that, we are taking advantage of 3D cell models of breast cancer previously developed by our group. We developed a culture strategy for co-culture spheroids of tumor cell lines together with stromal cells, encapsulated within alginate hydrogels, in stirred culture systems; we demonstrated that this strategy recreates features of the TME, such as deposition of ECM and secretion of soluble factors, e.g, cytokines. In a later stage of the project, we will validate our results in 3D cell models based on patient-derived tumor tissue, which are currently under optimization. Materials and methods: Firstly, we are evaluating Ab diffusion in empty capsules versus capsules containing breast tumor cell spheroids (BT474 cell line) and fibroblasts. Capsules are incubated with a fluorescent Ab for 2 hours and its diffusion is followed over time by fluorescence microscopy, ELISA and fluorimetry. These assays are being conducted at a range of pH values that mimic physiological variations in tumor/normal tissues. The capsule structure, with and without cells, is being characterized by electron microscopy. Results and discussion: Based on preliminary results, we expect that Ab transport across alginate capsules follows a dynamic profile characterized by a first phase of high rate of Ab diffusion to the interior of the capsule, due to the concentration gradient, followed by a progressively lower diffusion rate until the concentration inside the capsule becomes stable. Moreover, we expect that pH influences Ab transport due to charge differences in the molecule itself in response to pH changes, as well as due to the fact that the alginate matrix is itself charged. This work is a first step towards a better characterization of Ab diffusion within tumor mimicking microenvironments. Based on the experimental data generated we will implement an in silico model that can describe and predict antibody diffusion within microenvironments with specific characteristics.

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F20. Photochemical internalisation as an effective drug delivery method in 3D compressed collagen ovarian cancer models

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Introduction: Photochemical internalisation (PCI) is a light activated drug delivery method, which uses sub-lethal photodynamic therapy (PDT) to enhance the delivery of therapeutic agents that are prone to endolysosomal degradation to their intracellular target sites of action. The release of the endocystosed compound occurs when the vesicle ruptures due to activation of photosensitises by light and generation of reactive oxygen species. Since compressed 3D collagen models mimic the dense extracellular matrix of a human tissue more closely and also take into account the interactions between the cancer cells and the extracellular matrix, they are considered as ideal alternatives to 2D and animal models. Aim: To investigate the efficacy of PCI on compressed 3D collagen models of ovarian cancer. Materials and Methods: Compressed 3D collagen models of ovarian cancer were created using RAFT kit as well as SKOV3 and HEY (ovarian cancer) cells. The models were treated with either the photosensitiser (PS) (TPPS2a) alone or macromolecular toxin

(Dactinomycin) alone or a combination of both drugs and were incubated for 24 hours then exposed light. Cell viability was measured at 48 hours after illumination using Alamar blue assay and images were obtained using Live-dead assay and a fluorescence microscope to observe level of cell kill with PCI.Results and Discussion: PCI lead to a synergistic effect when using 1nM and 2nM dactinomycin compared to using PDT or dactinomycin alone with the PCI effect being slightly higher using 2nM dactinomycin compared to 1nM. For cells that were illuminated for 5 minutes PCI produced 3.1 fold higher efficacy in cell kill vs. PDT ($p< 0.001$) using 1nM dactinomycin and 4.4 fold higher efficacy vs. PDT ($p<0.001$) using 2nM dactinomycin in HEY cells. The same conditions produced 1.9 (1nM) and 3.1 (2nM) fold higher efficacy vs. PDT ($p<0.001$) in SKOV3 cells. These results have indicated that the compressed 3D cancer model is promising way to study PCI as well as demonstrating the potential of PCI as a minimally invasive method for treating ovarian cancer in the future. The significant difference between PCI and PDT results shows the synergistic effect produced with the combination of both TPPS2a and dactinomycin.

No conflict of interest

F21. Multistimulus-responsive nanoparticles for optimising sonodynamic therapy in prostate cancer

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Introduction: Sonodynamic therapy (SDT) employs ultrasound in combination with sensitizers for the production of cytotoxic reactive oxygen species and the subsequent confined ablation of tumours. Substantial pre-clinical studies have demonstrated the efficacy and targeting capability of this therapeutic approach. [1] However, SDT has yet to be fully characterised and appropriately exploited for the treatment of prostate cancer. Moreover, no therapeutic formulation has been developed that can increase intratumoral levels of sensitizers, achieving an improved therapeutic result based on SDT. Materials and Methods: In this study, a formulation based on multistimulus-responsive hematoporphyrin-containing nanoparticles that can accumulate in advanced prostate tumours and increase the therapeutic efficacy of SDT has been developed. The nanoparticles based on a co-polymer of poly-L-glutamic acid (PGA) and tyrosine were prepared using a modification of the self-assembly method described by Tarassoli et al. [2] The formulation is designed to respond to the microenvironment of advanced prostate tumours, such as the overexpression of the proteolytic enzymes, cathepsin-B and prostate-specific membrane antigen (PSMA), that can degrade the nanoparticles, reduce their size and improve cellular uptake. Results and Discussion: The sensitizer encapsulation efficiency was 11±0.41%. The mean nanoparticle diameter, determined using dynamic light scattering (DLS), was 110nm. The effect of cathepsin-B and pH on nanoparticle degradation over time was examined using DLS. Cellular uptake studies demonstrated that nanoparticle uptake was proportional to cathepsin-B expression and secretion by LNCaP, a PSMA-positive human prostate cancer cell line. In the absence of ultrasound, the formulation provided significantly increased cytotoxic effect at acidic cell culture conditions (pH 6.4), similar to the prostate tumour microenvironment, when compared to physiological pH conditions (pH 7.4). Sonodynamic treatment demonstrated ultrasound–induced cytotoxic effect only for the nanoparticle-treated cells, while toxicity of the formulation for the non-irradiated samples was minimal at physiological pH. These interesting properties exhibited by this formulation could potentially exploit the tumour microenvironment in evolving a more site-specific approach to SDT.

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No conflict of interest

F22. Chemical and biological characterization of chalcones and chromenes: exploitation as anticancer agents

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Introduction: Cancer is a devastating disease worldwide, with millions of diagnoses per year and many people living with this pathology. Breast cancer is the most frequent type of malignancy in women while male breast cancer represents, approximately, 1% of the total male diagnosed population. Due to the increasing cancer incidence, research in this area has been growing at the same rate. Thus, there is an urgency in discovering new drugs for cancer treatment. The chromene scaffold has been identified in several compounds with anticancer activity. The substitution pattern highly influences the activity and the mode of action, and the synthesis of new derivatives is an important element in the search for improved drug candidates. This work addresses the synthesis of new chromene derivatives with enhanced anticancer activity. Chalcone and chromene derivatives were isolated in good yield through clean reactions using innocuous solvents such as water and ethanol and effective aldol condensations leading to the isolation of highly pure compounds. The newly synthetized molecules were tested on cancer cells and a non-tumoral cell line. In the first screening, a range of 51 compounds was tested for cell survival on MCF-7 cells by the SRB assay, leading to the most promising compounds test in another breast cancer cell line (HS578t). The evaluation of cytotoxicity was performed on non-tumoral MCF-10 cells. Then, a selection of the eight chalcones and chromenes with the best anticancer activity had their IC50 determined for all three cell lines, and the molecules with better selectivity index proceeded to additional studies. Generally, compounds with halogenated aryl substituents presented enhanced activity compared to those with methoxy or methyl groups. More specifically, the bromine atom was often present in the bioactive molecules with greater selectivity that proceeded to the final assays showing to be promising candidates for further studies. Chalcones revealed to be bioactive compounds by inhibiting cell proliferation and disrupting cell membrane integrity. On the other hand, chromenes demonstrated a great capacity to inhibit cell migration and induce apoptosis, triggering cell death by G2/M cell-arrest.

No conflict of interest

F23. Influence of long-term treadmill training on mammary carcinogenesis

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Introduction: Mammary cancer is one of the most frequent cancers around the world. Mammary cancer development is associated with several risk factors, including lifestyle. This work aimed to evaluate the effects of long-term treadmill training on mammary carcinogenesis in a rat model of mammary cancer. Materials and Methods: Fifty female Sprague-Dawley rats of four weeks of age were randomly divided into four experimental groups: N-methyl-N-nitrosourea (MNU) sedentary (n=15), MNU exercised (n=15), control sedentary (n=10) and control exercised (n=10). At 50 days of age, animals from MNU groups received a single intraperitoneal injection of the carcinogen agent MNU. After this, animals from exercised groups were trained on a treadmill for 35 weeks (20 m/min, 60 min/day, 5 days/week). Mammary chains were weekly palpated to detect mammary tumors' development. Mammary tumors were monitored by ultrasonography, using B mode, Power Doppler, B Flow and contrast-enhanced ultrasound. At the sacrifice, serum samples were collected directly from the heart after animals' anesthesia. Mammary tumors were collected for histopathological analysis.Results and Discussion: As expected, animals from control groups did not develop any mammary

tumor. Lifelong treadmill training inhibited mammary carcinogenesis, by reducing inflammation (lower interleukin-6 and C-reactive protein serum levels in exercised groups, $p<0.05$), multiplicity (71 versus 50 mammary lesions in MNU sedentary and MNU exercised groups, respectively), burden (2.55 ± 1.44 tumors per animal versus 2.30 ± 1.42 tumors per animal) and malignancy (39 versus 21 malignant mammary tumors, $p<0.05$), and increasing cancer latency (10 versus 12 weeks). Long-term treadmill training also increased tumor vascularization as evaluated by Power Doppler (1.30% ± 0.29 versus 1.47% ± 0.27), B Flow (2.40%±0.52 versus 3.68%±0.88), contrast-enhanced ultrasound (area under the curve of 44.14±7.32 versus 44.59%±6.40), and immunohistochemistry (Vascular Endothelial Growth Factor immunoexpression of 55.91%±3.11 versus 66.04%±4.65). It is also worth to note that the exercise training increased the immunoexpression of estrogen receptor α (55.14% ± 13.26 versus 61.57%± 13.49 for MNU sedentary and MNU exercised groups, respectively) and β (70.06% ± 18.50 versus 70.70 ± 15.08), suggesting a better response of mammary tumors from exercised animals to hormone therapy.

No conflict of interest

F24. Cannabidiol (CBD) and Δ9-tetrahydrocannabiol (THC) inhibit aromatase and growth of ER+ breast cancer cells

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Introduction: Breast cancer is the main cause of cancer death in women worldwide. Oestrogen receptor positive (ER+) breast cancer totals around 75% of all breast cancer cases. These cancer cells overexpress the ER, to which oestrogens bind in order to trigger cell proliferation and tumour growth. Aromatase inhibitors (AIs) are the current first-line treatment for ER+ breast cancer in postmenopausal women, as they prevent oestrogen production and thus cancer progression (1). Unfortunately, despite its therapeutic efficacy, resistance to AIs can arise after prolonged treatment, so research into novel therapies is of utmost importance. The anticancer properties of phytocannabinoids derived from the Cannabis sativa plant, have already been shown in various cell lines and tumour types (2). Materials and Methods: We studied the anti-cancer properties of cannabidiol (CBD) and Δ9-tetrahydrocannabiol (THC), the abundant phytocannabinoids in C.sativa, on a human ER+ breast cancer cell line that overexpresses aromatase (MCF-7aro). To do so, we explored the effects on aromatase activity (radiometric assay) and its expression (Western-blot), cell viability (MTT/LDH assays), cell cycle progression (flow cytometry), ROS production and mitochondrial membrane potential (ΔΨm) (fluorimetric assays), caspase-7 activity (colorimetric assay) and cell morphology (Giemsa/Hoechst staining). Results and Discussion: CBD and THC have anti-aromatase activity in MCF-7aro cells, whilst also reducing its expression. Both cannabinoids decreased cell viability and disrupted cell cycle progression, without causing LDH release. Moreover, CBD and THC caused chromatin condensation in MCF-7aro cells and induced the apoptotic process. Curiously, neither cannabinoid increased ROS production and only CBD reduced ΔΨm. Thus, this work shows that these cannabinoids have the ability to inhibit aromatase and MCF-7aro cell growth, by impairing cell viability and cell cycle progression, and causing apoptosis independent of ROS. It also suggests that these cannabinoids could be potential AIs for use as a therapy for this type of cancer, although further studies are required. Acknowledgments: Amaral C (SFRH/BPD/98304/2013) Augusto T (BD/128333/2017) grants; Shiuan Chen (Beckman Research Institute, USA) for MCF-7aro cells. References: (1) Sobral AF et al. J Steroid Biochem Mol Biol. 2016; 163:1-11. (2) Fonseca BM et al. Rev Physiol Biochem Pharmacol. 2017; 173:63-88.

No conflict of interest

F25. The role of exemestane in bone homeostasis: the in vitro effects on osteoblasts and osteoclasts

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Introduction: Around 75% of all breast cancer cases are estrogen receptor-positive (ER+). Aromatase inhibitors (AIs) are the current first-line treatment for ER+ breast cancer in postmenopausal women. Exemestane (Exe) is a third-generation steroidal AI used in clinic, which binds irreversibly to aromatase and inhibits growth of breast cancer cells [1]. It has been suggested that Exe is the AI that has less adverse effects in bone loss, though, recent clinical trials showed that, in postmenopausal women, Exe enhanced osteoporosis and osteopenia [2]. Thus, as the impact of Exe in bone homeostasis is not fully clarified and bone health is an emerging concern in breast cancer therapy, in this study it was explored the effects of Exe in osteoblastogenesis and osteoclastogenesis. Materials and Methods: It was used an osteoblast-like cell line, MG-63, treated with Exe. The effects on cell viability (MTT/LDH assays), the alkaline phosphatase (ALP) activity (colorimetric assay) and the expression of osteoblast growth-related genes ALP, COL1A1, SPARC, and RUNX2 (qPCR) were explored. As osteoclast precursor cells, it was used human peripheral blood nuclear cells (PBMC) stimulated with M-CSF and RANKL. The effects of Exe on DNA synthesis and caspase-3/7 activation (fluorescence assays), tartrate-resistant acid phosphatase (TRAP) activity (colorimetric assay) and on the expression of osteoclast functional genes TRAP, CATK and CA2 and the osteoclast- differentiation/activation factors, c-myc and c-src, were investigated. Results and Discussion: Results indicate that Exe stimulates proliferation of MG-63 cells, increases ALP activity and the expression of ALP, COL1A1 and SPARC genes. Moreover, Exe in osteoclastic cells decreases DNA synthesis and TRAP activity, promotes caspase-3/7 activity and down-regulates the expression of TRAP, CA2, c-myc and c-src genes. Thus, this study suggests that Exe stimulates osteoblastogenesis and prevents osteoclastogenesis in vitro, indicating a potential protective effect on bone remodelling. These results may help to clarify the role of Exe in bone homeostasis. Acknowledgments: to FCT for Amaral C (SFRH/BPD/98304/2013) grant and financial support (UID/MULTI/04378/2013-POCI/01/0145/FEDER/007728). References: [1] Amaral C. (2012). PLoS ONE; 7(8): e42398. [2] Sobral A.F. (2016) J Steroid Biochem Mol Biol.; 163:1-11.

No conflict of interest

G1. P-cadherin promotes breast cancer stem cell anoikis resistance by stimulating glycolytic behavior and lowering oxidative stress

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Introduction: Breast cancer stem cells (BCSC) exhibit a pro-glycolytic metabolism, allowing them to decrease oxidative stress, escape anoikis and survive in circulation. P-cadherin (P-cad) is a poor prognosis factor in breast cancer, associated with hypoxic, glycolytic and acidosis markers. Still, P-cad enriched populations, with increased stem-like properties, are more likely to exhibit increased glycolysis and to survive to metabolic-driven pH alterations. The aim of this work was to evaluate if P-cad expression was responsible for the metabolic program of BCSC, acting as an antioxidant and enhancing their survival in circulation by promoting anoikis-resistance. Materials and Methods: Using a siRNA-mediated approach, we silenced the expression of P-cad in breast cancer cells and evaluated the glycolytic behavior, using the Seahorse XFe96 Extracellular Flux Analyzer (Seahorse Bioscience). ATP and ROS content were evaluated using luminescence and fluorescence, with CellTiterGlo, DCHF-DA and MitoSoxRed, respectively. Western blot, zymography and IHC was used to evaluate ROS scavenging systems and PDKs mRNA levels were assessed by qRT-PCR. Results and Discussion: We found that P-cad silencing decreases the extracellular acidification rate, as well as the ATP content of breast cancer cells. Interestingly, P-cad expression modulates the levels of the pPDH, through the modulation of PDK1/3, being probably the mechanism by which P-cad promotes the glycolytic behaviour of BCSC. Moreover, DCA, a metabolic modulator that deviates the glycolytic phenotype of BCC, induces anoikis preferentially in P-cad enriched BCSC. Finally, we also demonstrated that P-cad modulates the oxidative stress of breast cancer cells since its downregulation decreases the levels of ROS, by the upregulation of scavenging systems, such as SOD1 and SOD2. Importantly, this association was validated by IHC in primary invasive breast carcinomas, where an enrichment of SOD2 expression was found in P-cad overexpressing breast carcinomas. Taking together, we demonstrate for the first time, that P-cad is responsible for the glycolytic behavior as well as for the oxidative stress of BCSC, via pPDH/PDK axis and SODs, respectively. The metabolic role attributed to P-cad in this work suggests that this molecule is likely to promote BCSC survival in circulation and metastasis, being a possible player of therapeutic resistance of breast cancer patients.

No conflict of interest

G2. New insights into how cancer cells regulate glucose uptake by protein phosphorylation

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Introduction: Cancer cells require increased amounts of glucose to sustain their proliferation and upregulate plasma membrane expression of glucose transporter GLUT1. In insulin responsive cells, glucose uptake requires previous phosphorylation of TBC1D4, a negative regulator of endosomal GLUT traffic. Previous work published by the host lab has discovered that protein kinase WNK1 can also phosphorylate TBC1D4 and promote the translocation of GLUT1 to the cell surface. In vitro, WNK1 also phosphorylates the homologue TBC1D1 for which a role in cancer cell glucose uptake is not known. The extent to which WNK1 and both TBC1D proteins contribute to glucose uptake in cancer cells is not understood but its characterization is required for a systems--based understanding of glucose metabolism. Materials and Methods: In order to characterize the role of protein kinase WNK1, various colorectal cancer cell lines were first cultivated in the presence of different glucose concentrations. The amount of GLUT1 at the cell surface was compared under these conditions and the effect of depleting WNK1 expression by siRNA determined. For selected conditions, key cell cycle or apoptotic marker proteins were analyzed by Western blot in order to relate the role of WNK1 glucose--dependent cell growth and survival. Results and Discussion: WNK1--depleted cells cultured in low glucose medium showed higher apoptotic and cell--cycle arrest phenotypes. Concerning the key phosphorylation events involved in the regulation of GLUT1, mass spectrometry analysis revealed the WNK1--specific serine phosphorylation sites both in TBC1D1 and TBC1D4. Cell surface biotinilation assays shown Phospho--mimetic and non--phosphorylatable mutants modulate GLUT1 expression levels at plasma membrane. The mutants are currently being tested for their ability to regulate glucose metabolism by glucose uptake assays. Together, these studies will elucidate the molecular details regulating the translocation of glucose transporters in cancer cells and have the potential to identify novel therapeutic targets.

No conflict of interest

G3. 17β-Estradiol Stimulates the Glycolytic Metabolism of LNCaP Prostate Cancer Cells

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Introduction: Cancer cells have the characteristic ability of reprograming metabolism in order to enhance proliferation, growth, survival and maintenance. Among other metabolic adaptations, cancer cells display a preference for lactate production through aerobic glycolysis instead of the complete oxidation of glucose in the mitochondria, the so-called Warburg effect. Previous work of our research group has shown that androgens play a role driven the changes in glycolytic metabolism of prostate cancer (PCa) cells associated with progression of disease. Despite the well-known role of androgens in promoting development and progression of PCa, and more recently as metabolic regulators, the estrogens actions are almost unknown. Reports exist describing the action of estrogens controlling the metabolism of breast cancer, which lead us to hypothesis that these hormones may have a role modulating the metabolism of PCa cells. The present work aimed to investigate the effect of 17β-estradiol, the most potent estrogen, in regulating the glycolytic metabolism of PCa cells and identify the metabolic pathways altered in association with the progression of disease. Materials and Methods: The human neoplastic and androgen-responsive cancer cell line LNCaP was maintained in culture and treated with a range of 17β-estradiol (E2) concentrations (0,1, 1, and 100 nM) for 48 hours. After treatment, cells were harvested for protein extraction, and the culture medium of E2-treated and untreated cells was collected for measurement of extracellular metabolites. The concentration of glucose and lactate in the culture medium was assessed by means of spectrophotometric analysis using commercial kits. The enzymatic activity

of lactate dehydrogenase (LDH) was measured spectrophotometrically. Phosphofructokinase-1 (PFK1) and LDH expression was analyzed using Western Blot. Results and Discussion: The results obtained showed increased glucose consumption and lactate production in E2-treated LNCaP cells. The enhanced glucose consumption and lactate export were underpinned by the increased expression of PFK1 and LDH, and by the augmented activity of LDH, which support the role of E2 stimulating the glycolytic profile of LNCaP cells. These findings implicate estrogens in the enhancement of the glycolytic metabolism in LNCaP PCa cells and stimulate future work to investigate this metabolic relationship in conditions of estrogenic (de)regulation, e.g. obesity-associated hyperestrogenism.

No conflict of interest

G4. VEGFR-2-mediated reprogramming of mitochondrial metabolism regulates the sensitivity of acute myeloid leukemia to chemotherapy

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Introduction: Metabolic reprogramming is central to tumorigenesis, but whether chemotherapy induces metabolic features promoting recurrence remains unknown. We established a mouse xenograft model of human acute myeloid leukemia (AML) that enabled chemotherapy-induced regressions of established disease followed by lethal regrowth of more aggressive tumor cells. Human AML cells from terminally ill mice treated with chemotherapy (chemoAML) had higher lipid content, increased lactate production and ATP levels, reduced expression of PPARG coactivator 1α (PGC-1α), and fewer mitochondria than controls from untreated AML animals. These changes were linked to increased vascular endothelial growth factor receptor 2 (VEGFR-2) signaling that counteracted chemotherapy-driven cell death; blocking of VEGFR-2 sensitized chemoAML to chemotherapy (re-)treatment and induced a mitochondrial biogenesis program with increased mitochondrial mass and oxidative stress. Accordingly, depletion of PGC-1α in chemoAML cells abolished such induction of mitochondrial metabolism and chemosensitization in response to VEGFR-2 inhibition. Collectively, this reveals a mitochondrial metabolic vulnerability with potential therapeutic applications against chemotherapy-resistant AML.

No conflict of interest

G5. Lactate modulates epigenetic mechanisms contributing to kidney cancer progression

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Introduction: Metabolic reprogramming is a recognized cancer hallmark which plays a critical role in cancer development and progression. Kidney cancer is characterized by a glycolytic phenotype (Warburg effect) with increased lactate production, which is exported to the microenvironment through monocarboxylate transporters (MCTs). Recent studies demonstrate a role of lactate in tumor progression and therapy resistance. Several metabolites were previously reported as important modulators of epigenetic mechanisms, and histone deacetylases activity inhibition by lactate was recently hypothesized. Thus, understand lactate's role in epigenome regulation in high glycolytic tumors is remarkably relevant to find new therapeutic strategies. Materials and Methods: MCT1, MCT4, HIF-1α and VHL expression was characterized in 2 normal epithelial kidney cell lines (HEK-293 and HK-2) and 4 renal cell carcinoma lines (769-P, Caki-1, Caki-2 and ACHN) by RT-qPCR, western blot analysis and immunofluorescence. Then, the effect of lactate in enzymes involved in

epigenetic modifications was also evaluated and respective cellular localization was assessed by immunofluorescence. Finally, consequent alterations on cell migration and aggressiveness were performed. Results and Discussion: Class III histone deacetylases (Sirtuins) were found to be downregulated in all kidney cell lines treated with lactate. As a result, increased histone acetylation was observed. Furthermore, lactate was associated with increase cell migration and tumor aggressiveness markers. Hence, our preliminary results suggest that lactate regulates Sirtuins' family functions and might be implicated in cancer progression and aggressiveness.

No conflict of interest

H1. Decellularised Matrices: a 3D model to decipher the Extracellular Matrix-Cell crosstalk

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Introduction: Tissue homeostasis relies on the correct extracellular matrix (ECM) composition and organization. In cancer, ECM deregulation is a major molecular switch in the activation of multiple signalling pathways that can contribute to cell transformation, cancer progression and therapy resistance. However, little is known regarding the key ECM components and associated molecular pathways required to convey these features. To evaluate the influence of the ECM components and ECM-associated molecules on the selection of more aggressive clones, we have established a 3D decellularized ECM model of the human gastric mucosa. This will provide a 3D microenvironment that mimics the natural niche for cell growth and function. Materials and Methods: Both normal and tumour samples were derived from gastric cancer patients´ surgical resections. Decellularisation was achieved through sequential incubations in hypotonic buffer and a detergent solution, ending with a DNAse treatment. Normal and tumour samples (native and decellularized) were histologically characterized by Hematoxylin and Eosin (HE) or Masson's Trichrome (MT) staining. DNA content was assessed by DAPI staining and quantification. Immunohistochemistry of ECM proteins (collagen I, collagen IV, laminin, and fibronectin) was also performed. Complementary analysis of collagen distribution was done by transmission electron microscopy (TEM). In terms of biomechanics, we have analysed the viscoelastic properties of native and decellularized samples using a rheometer. Results and Discussion: We confirmed the efficiency of decellularisation as neither any defined nuclei nor diffuse nuclear material were detected. Further, our decellularized samples displayed a decrease in DNA content above 99.5%. HE and MT staining showed the absence of cytoplasmic material, with the latter demonstrating the maintenance of a collagen-rich matrix following decellularisation. Main ECM components analysed were also maintained in normal and tumour decellularized matrices, although their organisation is partially lost when compared to that of the native tissue, where these proteins present an organised distribution surrounding the glands. TEM analysis revealed that tumour matrices have increased deposition of collagen fibres, which is consistent with the rheological analysis indicating that tumour matrices are stiffer than normal ones. Moreover, decellularised matrices preserve the viscoelastic properties of the native tissue.

No conflict of interest

H2. Leukemic T cells stimulate NF-κB signaling in stromal cells through lymphotoxin-β receptor stimulation

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy arising from T-cell progenitors. The molecular crosstalk between thymocytes and stromal cells is key for T-cell development. Although T-ALL cells require stromal cell support to be maintained ex vivo, it is unclear how thymic microenvironmental cues support T-ALL. We previously showed that stromal lymphotoxin-β receptor (LTβR) favors thymic T-ALL development in Eμ-TEL-JAK2 transgenic mice. Mouse leukemic cells expressed lymphotoxin (LT) proteins and T-ALL development was impaired in Eμ-TEL-JAK2 mice lacking the Ltbr gene or treated with an LT antagonist. These results suggest that the LT-LTβR signaling axis mediates the crosstalk between malignant and non-malignant cells, thus favoring leukemogenesis. To test whether LT-expressing leukemic cells can activate LTβR in stromal cells we use an in vitro co-culture system. Since the main signaling pathway activated by LTβR is that leading to NF-κB transcription factor activation, we have generated luciferase reporter cell lines, by transducing LTβR-expressing stromal cell lines (NIH3T3 fibroblasts and MS5 bone marrow stromal cells) with a lentivirus carrying the luciferase reporter gene linked to an NF-κB promoter. The validation of the in vitro reporter cellular system was performed by LPS and agonist anti-LTβR treatment. For co-culture assays, primary leukemic cells from Eμ-TEL-JAK2 mice were seeded on top of reporter stromal cells. For LT-blocking experiments, a soluble LTβR-Fc fusion protein was used. Our results demonstrate that LPS and anti-LTβR activate the NF-κB-luciferase reporter in both cell lines. More importantly, mouse leukemic cells activated the NF-κB reporter in the MS5 and NIH3T3 cells. Showing that NF-κB activation was mediated through LTβR stimulation, luciferase activity in co-cultures was blocked by soluble LTβR-Fc protein. In addition, NF-κB reporter induction by co-cultured leukemic cells was impaired in LTβR-deficient mouse embryonic fibroblasts. We are currently generating LTβR knockout (KO) in MS5 and NIH3T3 stromal cell lines by CRISPR/Cas9 which will also carry the NF-κB-luciferase reporter. The LTβR-KO and WT stromal cells co-cultured with leukemic cells will be sorted for RNA-Seq analysis to identify the specific LTβR-dependent transcriptional program.

No conflict of interest

H3. High systemic cholesterol favors tumor cell intravasation by promoting a vascular mimicry phenotype

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Introduction: High systemic cholesterol levels have been positively correlated with tumor size and progression to metastasis in a variety of tumor models. While some of the molecular mechanisms through which systemic cholesterol leads to tumor growth have been identified, less is known about how cholesterol interferes with the formation of metastasis. Using an orthotopic model of breast cancer (4T1 cells) and a high cholesterol diet to rise cholesterol levels in circulation, we have been able to observe an increase in the number of circulating tumor cells in mice fed with the high cholesterol diet. This occurred at early time points of tumor progression and was not correlated with tumor size. Such data suggest that, apart from increasing tumor cell proliferation, a high cholesterol diet also favors an early event of the metastatic cascade that culminates with increased tumor cells in the peripheral blood. To identify the specific cellular and molecular mechanisms taking place here, we performed in vitro tests in which we exposed tumor cells to a cholesterol (LDL) enriched environment. As such we were able to show that, in high LDL conditions, tumor cell have increased capacity of transmigrating through endothelial monolayers. Further examination on this, using time lapse microscopy, shows that before transmigrating, in the presence of LDL, tumor cells intercalate with endothelial cells in higher frequency than cells in control conditions. This “vascular mimicry”-like phenotype in which tumor cells are able to intercalate with endothelial cells in tumor blood vessels in order to increase tumor perfusion, has been recently shown to contribute to the entry of tumor cells in circulation. This requires the action of anti-coagulants such as Serpine2 and SLPI. Interestingly we have been able to show that LDL induces the expression of Serpine2 at the mRNA level. As such we are now testing the hypothesis that high systemic cholesterol levels increase the ability of tumor cells to develop a vascular mimicry phenotype and therefore intravasate and metastasize.

No conflict of interest

H4. Examining the tumor immune microenvironment in young women (<40 years) with breast cancer

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Background: Tumor-infiltrating lymphocytes (TILs) have prognostic and predictive value in breast cancer but have been mainly evaluated in post-menopausal patients (pts). Sex hormones can modulate the immune system. Differences in immune responses between female reproductive phases or in pregnancy are well characterized. However, the impact of pts age on immune response (IR) associated with BC is not known. TILs distribution and T-cell IR phenotype may be different in young BC pts (<40 years) given the unique characteristics of the hormonal milieu in which these tumors develop. In the 1st phase of this project, pts <40 years old, were analyzed regarding TILs and in the 2nd phase a matched post-menopausal cohort and IR phenotype will be evaluated. Aim: To examine TILs distribution according to the tumor biologic subtype in BC pts <40 years old. Methods: Medical records from pts <40 years old, diagnosed at our institution between 2012 and 2016, were reviewed, including pathologic data and TILs evaluated in HE stained slides (surgical specimens or core biopsies) and where reported as a % value according to the International TILs Working Group. Lymphocyte predominant BC (LPBC) was defined as cases with at least >50% of stromal lymphocytic infiltration. Results: Sixty-eight pts with a median age of 37.6 years were enrolled. Invasive carcinoma NST was the most frequent histological type (94%) and Luminal like was the most common subtype (n=49; 72%), followed by triple negative (TN) (n=10; 15%) and HER2+ve (n=9; 13%). Stage I comprised 40%, stage II 44%, stage III 15% and stage IV 1%. Neoadjuvant therapy was performed in 28 pts. BRCA1/2 testing was performed in 19 (28%): 7 pts harbored BRCA mutations. Three pts (5%) had pregnancy related BC (PRBC). The median TILs % score was 10% (IQR: 5-20%). TILs % was significantly different across the biologic subtypes (p<0.0004) being higher in TN (37.5%) in comparison with HER2+ve (12.3%) and Luminal type (13.8%) subgroups. LPBC phenotype comprised 13% of the cases, with significant differences between the biologic subtypes (p=0.0012): 50% (TN), 11% (HER2+), and 6% (Luminal). Median TILs % score was not significantly different (p=0.57) between the BRCA mutated (20.8%) and BRCA wild-type (16.6%) pts. PRBC (all Luminal like) presented a median TILs% of 40%. Conclusions: Our findings raise the hypothesis of a higher frequency of LPBC in young pts with TN BC as compared to historical data in post-menopausal pts. Interestingly in PRBC our results suggest higher levels of TILs in comparison with previously published data. Further investigation requires larger sample size. The phenotype of T-cell IR, variations in lymphocytic cell subsets (CD3, CD8 and FoxP3) and comparison with a matched post-menopausal cohort is ongoing.

No conflict of interest

H5. Microglia-Glioma Crosstalk Trigger the Dysruption of Blood-Brain Barrier Via IL-6/JAK/Stat Pathway

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Introduction: Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor, with an average life expectancy of 12-15 months. GBM is highly infiltrated by microglia (MG) that under the tumor microenvironment acquire an activation phenotype with tumor-supportive features that promote the tumor growth and invasiveness. Additionally, microglia activation and subsequent neuroinflammation seems to be involved in blood-brain barrier (BBB) dysfunction frequently observed in brain tumors, although the mechanism underlying this effect remain to be thoroughly clarified. Herein, we evaluated the effects of reciprocal interactions between MG and GBM cells in the integrity of brain endothelial cells (ECs). Material and Methods: Microglial BV-2 cells were co-cultered with U87 GBM cells in a transwell system during 48h. A monolayer of the human brain endothelial cell line hCMEC/D3 was exposed to conditioned medium of the co-culture (CM-CC). The transendothelial electrical resistance (TEER) and the macromolecular flux of 4 kDa-FITC and 70kDa-RITC across the ECs monolayer were measured. The intercellular junction proteins β-catenin and zonula occludens (ZO)-1 in ECs was analyzed by immunocytochemistry. Results and Discussion: The exposure of ECs monolayer to the conditioned medium harvested from the MG/GBM co-culture induced a decrease in the

TEER and an increase in permeability of both fluorescent dyes across the confluent ECs in relation to control cells. These effects were accompanied by a decrease in the expression of the intercellular junction proteins, namely in β-catenin and ZO-1 that are important elements of the intercellular junction structure. Moreover, the dynamic interaction between microglia and tumor cells triggered the release of interleukine-6 (IL-6) and consequent activation of JAK/STAT pathway. Interestingly, the blockade of this pathway with AG490 was able to prevent the ECs hyperpermeability. Overall, our results demonstrated that IL-6 released by MG-GBM interaction leads to barrier dysfunction through the activation of the JAK/STAT pathway. The modulation of this pathway may therefore constitute a potential therapeutic approach to attenuate the BBB disruption in glioma patients. Acknowledgments: Project PTDC/NEU-OSD/0312/2012 (COMPETE and FEDER funds); Pest-C/SAU/UI3282/2013-2014 and CNC.IBILI UID/NEU/04539/2013 with national funds PT2020/COMPETE 2020 and POCI (FCOMP-01-0124-FEDER-028417 and POCI-01-0145-FEDER-007440).

No conflict of interest

H6. Influence of KRAS activation in the colorectal cancer immunosurveillance escape

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Introduction: The immune system as a host defense system watches the cell growth and division, eliminating cells with antigens different from those present in healthy cells. However, some transformed cells have the capacity, through various mechanisms, to escape the immune system. Genomic instability and some mutations are pointed as possible mechanisms supporting the immunosurveillance escape, as is the case of KRAS mutation. This oncogenic mutation is present in about 30% of cases of colorectal cancer and confers to the tumor a greater potential for malignancy. It is known that KRAS mutant cancer cells regulate the recruitment, activation, and differentiation of immune cells, promoting tumor evolution by ensuring leakage to the immune system and increasing the proliferative potential. Few evidence highlights an association between a KRAS mutation and myeloid cells, mainly macrophages and neutrophils infiltration. However, the mechanism which determines this interaction remains unclear. Due to the growing knowledge of different immunosuppressive molecules, it became interesting to investigate if there is an alteration in these molecules related to the KRAS activation. Materials and Methods: In our work, a series of immunosuppressive molecules were analyzed by flow cytometry in a panel of KRAS mutant colorectal cancer cells in which KRAS was silenced by small interfering RNA. Results and Discussion: Preliminary results suggest that the silencing of the KRAS oncogene lead to the alteration of some molecules involved in the crosstalk with the immune system cells, such as macrophages. In conclusion, the KRAS activation seems to be capable to regulate the expression of surface markers which can regulate and suppress the immune response of the tumor infiltrated immune cells.

No conflict of interest

H7. Mutant KRAS mediates fibroblast-induced colorectal cancer cell invasion

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Introduction: KRAS is the most frequently mutated oncogene in colorectal cancer (CRC), being a potent initiator of tumorigenesis, a strong inductor of malignancy, and a predictive biomarker of non-response to anti-EGFR therapies. As such, extensive research has been done to exploit KRAS and its downstream signaling effectors as therapeutic targets. However, KRAS proved difficult to target, and inhibition of its signaling effectors has never resulted in significant clinical responses, highlighting the need for a better understanding of KRAS-associated

signals. Since the tumour microenvironment plays a key role in tumour aggressiveness, research on this area became an attractive alternative as new targets for therapy may arise from the study of cancer cell-microenvironment crosstalk. The aim of this study was to characterize, at the molecular and functional levels, the role of mutant KRAS in mediating CRC cells-fibroblasts crosstalk. Materials and Methods: Using fibroblasts-conditioned media (CM) as a chemoattractant, we performed matrigel invasion assays with KRAS mutant CRC cell lines in which we silenced KRAS using siRNA. Additionally, we performed ELISA assays to quantify the levels of fibroblasts-secreted factors and resorting to western blot we evaluated the expression of some cell surface proteins. Results and discussion: By performing in vitro invasion assays we observed that the CM promoted CRC cell invasion in a KRAS-dependent manner. Analysis of the CM for the detection of pro-invasive factors, revealed the presence of high levels of HGF. Accordingly, neutralization of HGF in the fibroblasts CM abrogated CRC invasion, and supplementation of control CM with HGF induced invasion in a KRAS-dependent manner. Additionally, we have also observed that KRAS regulates the expression of HGF receptor, C-MET, along with other C-MET co-receptors. In conclusion, our results show that KRAS may be an important modulator of response to fibroblasts-secreted factors that induce CRC cells invasion. Therefore, this work suggests that targeting of C-MET can be a useful tool to abrogate invasion of KRAS mutant tumours and sets a rational to test C-MET inhibitors in the treatment of KRAS mutant CRC patients, who currently lack effective therapeutic options.

No conflict of interest

H8. The importance of considering hypoxia on the macrophage-cancer cell interplay

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Introduction: Although hypoxia and macrophages are independently associated with tumor progression, and macrophages being recruited to hypoxic areas, there is scarce information about hypoxia influence on macrophage-cancer cell crosstalk. In most cases, neither experiments studying the interplay macrophage-cancer cell were performed in hypoxia, nor had the studies about hypoxia focused on macrophage-cancer cell interplay, resulting in tumor microenvironment understanding gaps. Our aim was to evaluate the impact of hypoxia on the macrophage-colorectal cancer cells interactions. Material and Methods: We started by analyzing the correlation of macrophage and hypoxia markers in patients, and proceed with monocultures or cocultures of cancer cells and macrophages under normoxia/hypoxia. The impact of hypoxia was evaluated on macrophage polarization, phagocytic activity, cytoskeleton organization, and on cancer cell invasion, invasion-associated signaling, and epithelial-mesenchymal transition markers. Metalloproteinases expression was evaluated on both populations. Results and Discussion: Bioinformatics analysis showed a significant association between CD68 and HIF1α expression in several cohorts. We found that hypoxia induced a decrease on macrophage CCR7 and CD163 expression, and an increase on MMP7, a decrease on their phagocytic activity. On cancer cells, hypoxia increased invasion, Erk1/2 activity, vimentin and Snail1 expression, decreasing c-Src

activity and fibronectin expression. Our results revealed that in CRC, hypoxia modulates macrophage-mediated cancer invasion and associated pathways, without major differences on macrophage polarization status.

No conflict of interest

H9. Stroma cells stimulate colorectal tumor cells to increase expression of tumor-promoting RAC1B

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Introduction: An inflammatory microenvironment is a tumor-promoting condition that provides survival signals to which cancer cells respond with changes in their gene expression. One key gene regulatory mechanism that responds to extracellular signals is alternative splicing. For example RAC1B, a RAC1 alternative splicing variant that we previously identified in a subset of BRAF-mutated colorectal tumours, was found increased in samples from inflammatory bowel disease patients or following experimentally-induced acute colitis in a mouse model. The main goal of this work is to determine the pro-inflammatory signals that lead to increased RAC1B expression in colorectal cells. Materials and Methods: Caco-2 colorectal cells were either grown as polarized cell monolayer on porous filter membranes and then co-cultured with different stromal cell lines (fibroblasts, monocytes and macrophages) for 48 h, or grown as cysts in 3D matrices. RAC1B expression was analysed by RT-PCR, Western blot and confocal fluorescence microscopy. Results and Discussion: Culture conditions for polarized 2D and 3D models were established as physiologically more relevant colon cell models. Co-culture experiments with polarized cells revealed that the presence of fibroblasts and/or macrophages induced a transient increase in RAC1B protein levels in the colorectal cells, accompanied by a progressive loss of epithelial organization. The cytokines secreted by fibroblasts and macrophages are currently being identified. Our data indicate that extracellular signals from stromal cells can affect gene expression in colorectal cancer cells. The observed increase in alternatively spliced RAC1B will help to understand the tumor-promoting effect of inflammation and identify novel therapeutic targets.

No conflict of interest

H10. FUNCTIONAL ANALYSIS OF BONE MARROW NICHES DRIVING DISSEMINATED CANCER CELL DORMANCY

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Introduction: Cancer patients treated for their primary lesions often display remarkably prolonged disease-free periods before developing metastasis. This phenomenon can be observed in the bone marrow (BM) where solitary disseminated tumor cells (DTCs) persist in a dormant state. If dormant cells could be prevented from awakening and surviving, metastasis could be prevented or significantly delayed. We published that DTCs in the mouse BM enter dormancy in response to at least TGFβ2, which induces cancer cell dormancy through a high p-p38/p-ERK ratio and p27 upregulation. Similarly, BMP7 induces cancer cell dormancy by activating p38 and increasing the expression of p21. However, the identity of the BM-resident host cells producing these signals has remained elusive. The hematopoietic stem cell (HSC) niche was described as indispensable for maintaining HSC dormancy, self-renewal and expansion. Nestin+ peri-arteriolar stromal cells enriched in mesenchymal stem cells (MSC) were shown to induce HSC dormancy through TGFβ signaling. We hypothesized that Nestin+ MSCs, could induce and maintain dormancy of DTCs in the BM through high TGFβ2 and BMP7 production. Materials and methods: We used transgenic mice where Nestin+ MSCs were labelled with GFP driven by the nestin

promoter. We sorted GFP+ vs GFP- cells to characterize the effect of these cells in co-culture on different cancer cell types. Current efforts are aimed to determine the spatial relationship between DTCs, TGFβ2 and BMP7 niches and Nestin+ MSCs, as well as in functionally analyze DTCs in mice with depletion of Nestin+ MSCs (NG2-Cre-iDTR), and depletion of TGFβ2 specifically from the Nestin+ MSCs (NG2-Cre-TGFβ2loxp). Results and Discussion: We found that Nestin+ MSCs express higher mRNA levels of TGFβ2 and BMP7 when compared to Nestin- cells. Furthermore, the conditioned media from BM derived from NG2-Cre-ER-iDTR (with reduced Nestin+ MSCs) mice show reduced levels of TGFβ2 than the mice that has intact Nestin+ MSCs, suggesting that Nestin+ MSCs are a source of TGFβ2 and BMP7. Additionally, in vitro 3D co-cultures of cancer cells with Nestin+ or Nestin- cells revealed that Nestin+ MSCs suppressed growth of human HNSCC and mouse BrCa cells keeping them as single or two-cell clusters in the absence of cell death, through SMAD, p38 and p27 activation. TGFβ2, BMP7 or BM-CM from TGFβ2+/+ mice also suppressed BrCa cell growth through SMAD, p38 and p27 activation, while this effect was partially reverted with BM-CM from TGFβ2+/- mice. Taken together, our data suggests that Nestin+ MSCs are a source of TGFβ2 and BMP7 dormancy-inducing cues. Our studies provide a novel link between HSC and DTC dormancy regulation in bone marrow niches, where dormant DTCs are found.

No conflict of interest

H11. Monocarboxylate transporters expression is influenced by different extracellular conditions determining the effect of 3-bromopyr

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Introduction: Monocarboxylate transporters (MCTs) play a vital role in the glycolytic metabolism in cancer cells, exporting lactate by a proton symport mechanism, maintaining intracellular pH homeostasis and contributing for tumor microenvironment acidification and aggressiveness. 3BP is an analogous of lactate with anti-tumor properties. 3BP uptake occurs via MCTs, acting as a glycolytic inhibitor with already identified targets, including hexokinase (HK) II and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Indeed, 3BP induces energy depletion and cell death. As tumor cells overexpress MCTs, they can be used as a trap to mediate the uptake of compounds as 3BP. However, the tumor microenvironment may influence the expression of MCTs and consequently affect the toxic effect of 3BP. In this work, we aimed to characterize the effect of 3BP in different colorectal cancer (CRC) cell lines by exploring 3BP cytotoxicity and MCT1 and MCT4 expression upon different extracellular stimuli, including extracellular pH (pHe), glucose and oxygen levels as well as short-chain fatty acids (SCFAs) exposure. Materials and methods: Viability assays: The IC50 of 3BP was determined in basal conditions and in the different conditions: hypoxia, starvation, different pHe and exposure to SCFA. HCT-15 cells were incubated during 24 h with medium containing 200 µM of CoCl2 (chemical hypoxia), free-glucose medium (starvation), complete medium (w/o bicarbonate) adjusted with HEPES buffer to pH 6.6 or 7.4 or with complete medium containing butyrate (10 mM). When cells were incubated with medium containing lactate (50 mM) or acetate (20 mM), cells were incubated during 48 h. After that, cells were exposed to different concentrations of 3BP for 16 hour and cell viability was determined after this period of time by the SRB assay. Metabolites quantification: HCT-15 cells were exposed to different extracellular conditions and lactate and glucose were measured in extracellular medium. Expression assays: MCTs expression was evaluated by Western blot assays. Protein extracts of HCT-15 cells lines, incubated in the different conditions tested, were used. Result/Discussion: In this work, we tested the effect of 3BP in three different CRC derived cell lines: HCT-15, Caco-2 and HT-29. HCT-15 cells showed to be the most sensitive cell line to 3BP and also the one that presented the highest basal expression of both MCT1 and CD147, a protein involved in the proper expression and activity of MCT1 and MCT4 at cell surface. Glucose starvation and hypoxia induced an increased resistance to 3BP in HCT-15 cells, in contrast to what happens with at acidic pHe. However, no association with MCT1, MCT4 and CD147 expression was observed, except for glucose starvation, where a decrease in CD147 (but not of MCT1 and MCT4) was detected. Butyrate and acetate (but not lactate) exposure increased the expression of MCT4 (but not MCT1) and CD147. Additionally, it was observed that the metabolic profile was affected by the

different extracellular conditions. The overall results suggest that MCTs influence 3BP effect in CRC cells, although they are not the only player in its mechanism of action.

No conflict of interest

I1. Castrate-resistant prostate cancer cells display differential response to imatinib dependently on glycaemic conditions

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Introduction: Castrate-resistant prostate cancer (CRPC) is an aggressive stage of prostate cancer (PCa) characterized by its androgen-independent growth. Other features of CRPC are the hyperglycolytic profile and resistance to apoptosis. Although new therapies have emerged recently, the therapeutic options for CRPC remain restricted and display limited duration of clinical and survival benefits. Imatinib, used for treatment of leukemias and gastrointestinal tumors, is a chemotherapeutic drug that inhibits the tyrosine kinase activity of c-KIT receptor and others. Previous work of our research group showed that imatinib differently affected CRPC cell lines models concerning the regulation of cell cycle, apoptosis, and angiogenesis. On the other hand, experimental evidence indicates that glycaemia influences the therapeutic response in PCa, which raises the curiosity about the effect of imatinib in conditions of different glucose availability. Materials and Methods: CRPC cells, DU145 and PC3, were treated with 20 µM imatinib in the presence of 30 mM (hyperglycaemic) or 5 mM (hypoglycaemic) glucose for 48-72 h. The MTS assay was used to assess cell viability, and the enzymatic activity of caspase-3 was included as an indicator of apoptosis. The expression of cell cycle, apoptosis, and metabolic regulators was assessed by Western Blot analysis. The content of glucose and lactate in cell extracts and culture medium was measured using biochemical assays. LDH activity was determined spectrophotometrically. Results and discussion: Imatinib decreased the viability of DU145 and PC3 cells under hyperglycemic conditions only. The diminished cell viability was accompanied by the altered expression and activity of apoptosis regulators, like Bcl-2. Caspase-3 activity was augmented in the imatinib-treated group in high-glucose conditions. Altogether, the results obtained in both CRPC lines showed that imatinib is more effective inducing apoptosis and decreasing cell viability in conditions of hyperglycemia. Glucose consumption and lactate production were increased upon treatment with imatinib, concomitantly with the altered expression of glucose and lactate transporters. This work demonstrated that imatinib effects controlling the fate of CRPC cells were potentiated in high glucose availability. Imatinib treatment also stimulated the glycolytic metabolism of CRPC cells, which can be a mechanism favouring the metabolic reprogramming and survival adaptation of cancer cells.

No conflict of interest

I2. Counteract Cancer´s next Move: TRIB2-mediated AKT activation is a mechanism of therapy resistance

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Introduction: Melanoma is the most aggressive form of skin cancer resistant to all standard therapies. Drug resistance is the major cause of treatment failure in melanoma. Our lab has identified TRIB2 as an oncogene which is dramatically overexpressed in malignant melanoma. We previously demonstrated that TRIB2 acts as a suppressor of FOXO transcription factors, the major transcriptional mediators of the PI3K/AKT pathway. As FOXO proteins are involved in the action of several anticancer drugs we hypothesized that TRIB2-mediated FOXO suppression can lead to drug resistance. In this study we show that TRIB2 indeed confers resistance to drugs used in the clinic by binding to and activating AKT. Importantly we show that this novel mechanism of drug resistance has clinical relevance. Materials and Methods: Using paired isogenic cell lines harboring silenced or overexpprese d TRIB2 and corresponding xenografted animal models, we analyzed the sensitivity to several drugs relevant in the treatment of melanoma such as dacarbacine, gemcitabine, PI3K, AKT and mTOR inhibitors. To examine the functional importance of FOXO in TRIB2-dependent cell line resistance we used RNAi mediated silencing of TRIB2. We also mapped the domain of the TRIB2 protein responsible for drug resistance using several TRIB2 mutants. qPCR was used to determine the expression of p53 and FOXO target genes, Western blot analysis and immunohistochemistry was employed to monitor expression and activation status of components of the PI3K/AKT pathway. TRIB2/AKT interaction was analyzed by protein complementation assays and immunoprecipitation assays. Patient samples with full clinical histories were obtained from Department of Dermatology, Julius-Maximilians University, Würzburg, Germany. Results and Discussion: Our study shows that TRIB2 confers resistance to several drugs relevant for the treatment of melanoma and other tumor types providing a novel regulatory mechanism underlying drug resistance. As intrinsic and acquired resistance to all treatment modalities is the major cause of treatment failure in melanoma, the implications of the current work for clinical management of melanoma patients are extremely important.

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No conflict of interest

I3. Possible new therapeutic strategies to overcome resistance to MAPK inhibitors treatment in cutaneous melanoma patients

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Introduction: Cutaneous melanoma represents the deadliest form of skin cancer. Over the last few years, several therapies have been approved by Food and Drug Administration (FDA), including targeted and immunotherapies. However, the overall survival of patients did not improve. In 2011, FDA approved

vemurafenib, a selective oral BRAF-mutant inhibitor, for the treatment of unresectable or metastatic melanomas harbouring BRAFV600E mutations. Nevertheless, this therapy presents limitations due to the rapidly acquirement of resistance. In order to overcome the resistance to vemurafenib, in 2015, FDA approved the combination of vemurafenib with cobimetinib, an oral selective MEK inhibitor, for the treatment of melanomas harbouring BRAF mutations, which cannot be surgically removed or display metastization. There is still the need to develop new therapeutic modalities, in order to improve the survival of melanoma patients. The mechanisms of resistance to vemurafenib, which occur frequently in BRAFV600E melanoma patients, may be related with the deregulation of MAPK and PI3K/AKT/mTOR pathways and the presence of the Warburg effect in melanoma cells. Thus, we hypothesized that MAPK pathway inhibitors resistance can be overcome by the combination of vemurafenib with an mTOR inhibitor (everolimus) or with a metabolic modulator, dichloroacetate (DCA). In this work, the effects of the treatment with vemurafenib, cobimetinib, DCA and everolimus, either alone or in combination, were evaluated by the quantification of cell viability, using Presto Blue assay, in a cutaneous melanoma cell line sensitive to vemurafenib and in a cutaneous melanoma cell line resistant to vemurafenib, both harbouring BRAFV600E mutation. Our results suggest that the combination of vemurafenib with DCA can be a valuable approach for the treatment of melanoma patients. Funding was obtained from the project "Advancing cancer research: from basic knowledgment to application"; NORTE-01-0145-FEDER-000029; "Projetos Estruturados de I&D&I", funded by Norte 2020 – Programa Operacional Regional do Norte.

No conflict of interest

I4. CD44v6 expression modulates response to cisplatin treatment in gastric cancer cells

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Introduction: Gastric cancer (GC) is the 3rd leading cause of cancer related deaths worldwide with >80% of patients presenting advanced and/or unresectable disease. Conventional chemotherapy (mostly platinum-based) results in ~1 year overall survival. Poor patient survival is likely due to late-stage diagnosis and weak therapy-response. We hypothesized that some GC cell sub-populations display an intrinsic drug resistance phenotype and explored de novo expression of CD44v6-containing isoforms as candidate molecules in GC drug resistance. While CD44 standard isoform (CD44std, main hyaluronic acid receptor) is expressed in all gastric epithelial cells, the variant CD44v6 becomes de novo expressed in pre-malignant GC lesions and maintains its expression in ~70% of all GCs. CD44v6 has been associated with drug-resistance phenotypes in several cancers. Herein, we aim to understand the role of CD44v6 overexpression in response to cisplatin-based chemotherapy in GC cells. Materials and Methods: We established isogenic GC cell lines overexpressing CD44v6, CD44std, or the empty plasmid (Mock). We also depleted CD44v6 by RNAi in GC cell lines that endogenously express CD44v6. All cell lines were characterized for CD44 variants and/or potential signaling partners by RT-PCR, western blot, immunofluorescence and flow cytometry. The effect of cisplatin on cell survival was evaluated by SRB and Annexin-V assays. Results and Discussion: While CD44v6 overexpression led to increased cell survival in response to cisplatin, depletion of CD44v6 led to sensitization of GC cells to the same treatment. Isogenic CD44v6-expressing cells were then co-cultured with their non-expressing counterpart, treated with cisplatin and allowed to recover for 15 days. At the end, CD44v6-expressing cells represented >80% of the culture. These results indicate that CD44v6 is involved in GC cell overgrowth after cisplatin treatment. When investigating CD44v6-signaling partners that may trigger this behavior, we found a faster translocation to the nucleus of phospho-Stat3 in CD44v6-expressing cells than in non-expressing cells. Conclusion: Our findings highlight CD44v6 as a modulator of response to cisplatin treatment, acting through phospho-Stat3 in the

studied cell lines. Novel therapeutic strategies including depletion of CD44v6 and/or signaling partners, at the tumour site, may improve therapy response and survival in GC patients.

No conflict of interest

I5.

Selected for oral presentation – Symposium II

I6. Preliminary characterization of the PERK unfolded protein response branch in endocrine responsive and resistant cells

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Introduction: An increasing number of publications support the hypothesis that impairments in the unfolded protein response (UPR) trigger cancer in the breast(BC) and prostate (PC). Current knowledge on UPR function in endocrine response and resistance is based on acute exposure to different estrogen and androgen receptor agonists and antagonists. As the effect of agonists and antagonists at different time points leads to a variety of effects on UPR branch activation we aimed to understand how the diversity of responses are modulated by acute and chronic exposure in endocrine therapy resistant vs. sensitive BC and PC cell lines. Material and Methods: MCF74-hydroxytamoxifen(TAM) sensitive (MCF7S), MCF7 TAM resistant (MCF7R), LNCaP flutamide (FLU) sensitive and 22Rv1 FLU resistant cell lines were used. They were treated with the agonists 10nM 17β-estradiol and 10nM Testosterone or the antagonists 500nM TAM and 10nM FLU dissolved in 100% ethanol for 1, 3, 6, 12, 24 and 48 h. Cell proliferation was assessed by cell counting following 5 days treatment. Flow cytometry and Western blotting were employed to assess the expression of UPR proteins. Results and Discussion: In MCF7S and MCF7R cells a 3h agonist treatment upregulated BIP which was able to maintain inactive PERK/EIF2α branch. On the other hand, BIP increase was initially slower in PC leading to a small pPERK activation without EIF2α inhibition in response to TEST. Exposure of MCF7S cells to TAM for 12h lead to BIP induction and maintained inactive pPERK and pEIF2α. LNCaP cells treated with FLU for 1h showed an initially slower BIP induction and a small transient (1h-3h) pPERK increase possibly leading to reduced protein synthesis.Treatment of resistant cells with antagonists lead to a transient (6h-24h) BIP decrease and to a pPERK increase without EIF2α inhibition, suggesting the activation of other pPERK-dependent pathways.Agonist treatment do not inhibit protein translation in both BC and PC cells. Resistant cells seem to modulate UPR activation differently as increased EIF2α levels without its inhibitory phosphorylation may be related with the translation of proteins necessary to restore proteostasis and maintain survival. Acknowledgements: Dr. Julia Gee for kindly provide MCF7S and MCF7R cell lines; SFRH/BD/123821/2016; Institute for Biomedicine UID/BIM/04501/2013

No conflict of interest

I7. Characterization of TRIB2-mediated drug resistance

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Introduction: Intrinsic and acquired resistance to conventional and targeted chemotherapeutics is the fundamental reason for treatment failure for many cancer patients. As melanoma invariably acquire resistance to single-agent treatments, the identification of mechanisms underlying drug resistance has enormous clinical and economic value. The Link lab has discovered a novel mechanism of resistance to drugs in clinical trials/use

for melanoma therapy mediated by the kinase-like protein TRIB2. Importantly, TRIB2 confers resistance to the dual PI3K/PI3K inhibitor BEZ235 (Novartis). Data produced in the lab shows that TRIB2 expression leads to suppression of the FOXO and p53 target genes. In order to analyze the impact of TRIB2 on downstream transcriptional programs in an unbiased manner we performed RNA sequencing based transcriptomic analysis. Materials and Methods: In order to systematically survey the impact of TRIB2 on gene expression in the presence of BEZ235, we sequenced the transcriptome of isogenic U2OS cells with different TRIB2 status. RNA-Seq was performed in the Genomics Unit of the Centro Nacional de Investigaciones Cardiovascular (Madrid, Spain) using the Illumina Next Generation Sequencing platform. Differentially expressed genes (DEGs) were validated by RT-qPCR and Western-Blot for further evaluation of the candidate gene roles in TRIB2-mediated resistance. Results and Discussion: The specific role of candidate genes, is being evaluated by their ectopic expression in the context of high TRIB2 expression in the presence of the BEZ235, both by RNA-Seq and by RT-qPCR. Genes such as SOX2, essential for maintaining self-renewal and pluripotency in stem cells, SESN2 important in cellular homeostasis, MYCN, its amplification has been found to correlate with poor prognosis, and KRT14 known to increase cancer cell proliferation, were all upregulated by TRIB2. BEZ235-induced upregulation of the metastasis suppressor gene KiSS1 is reduced when TRIB2 is overexpressed. All these genes are candidate genes for TRIB2-mediated tumorigenesis and drug resistance and their role will be further evaluated. These preliminary results will allow us to investigate further how TRIB2 impact on downstream effectors and characterize their role in TRIB2 associated cancer phenotypes, such as on cell migration and apoptosis, leading for the discovery of new targets for therapy when TRIB2 is upregulated on those patients and standard chemotherapy does not work.

No conflict of interest

I8. RKIP protein induces HER receptors activation and positively modulate its pharmacological targeting in cervical cancer

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Introduction: Cervical cancer (CC) is estimated to account for 8% of the total cancer-related mortality in women worldwide, for who platinum based chemotherapy is just palliative. Recently, we found that HER receptors (EGFR and HER2) are good targetable proteins for fighting CC, however, resistance to that drugs remains an enormous clinical problem for other tumor types. Thus, aiming to anticipate the putative molecular mechanisms of resistance, we hypothesized herein that RKIP could be modulating cells response to HER targeted therapies given its role in the regulation of important signaling pathways in oncogenesis and therapy prediction, such as MAPK. Using a panel of CC cell lines, we knocked out RKIP using CRISPR/CAS9 technology to study RKIP role in HER signalling cascade activation, by western blot, and in the modulation of response to HER-targeted drugs (AST1306, lapatinib and afatinib), by cytotoxic assays. Additionally, in vivo CAM assay was used to validate the in vitro cytotoxic assays. Finally, a series of 202 CC human tissues and an in silico analysis was used to further explore the clinical potential of the findings. It was demonstrated for the first time that RKIP expression decrease whenever HERs activation increases and, in the other way around, RKIP knockout induces HER receptors overactivation. In agreement, we found in human samples that RKIP low expressing tumors have significantly high expression of total EGFR, a finding that was validated in silico. Importantly, and in contrast to what was expected, we observed both in vitro and in vivo that absence of RKIP expression is a positive predictor of response to HER inhibitors, an association that was found only in KRAS mutant cell lines that showed to be RKIP-dependent for signaling. Finally, in order to re-enforces the translational potential of our work, we validated RKIP low expression as worse prognostic marker in CC patients. The good news, due to the results obtained here, is that those patients are probably the ones that will most benefit from an anti-HER therapy.

No conflict of interest

Topic J
TUMOUR IMMUNOLOGY

J1. Monitoring immunological status in immune checkpoint inhibitor therapy of stage IIIB/IV non-small cell lung cancer

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Introduction: This prospective pilot study intended to evaluate the immunological status of 15 patients with stage IIIB/IV non-small cell lung cancer. Flow cytometry was used to analyze peripheral blood from three groups of stage IIIB/IV NSCLC patients: newly diagnosed (DX), starting immunotherapy after chemo (CT), undergoing immunotherapy (IMT) with PD-1/PD-L1 blockade agents. Blood donors were used as controls (CTRL). Most important finding consists in myeloid-derived suppressor cells (MDSCs) significant increase in all NSCLC patients compared to CTRL, particularly monocytic MDSC (M-MDSC) defined as Lin-HLA-DR-CD33+CD11b+CD14+CD15-. Similarly, type 2 myeloid dendritic cells (mDC2), defined as Lin-HLA-DR+CD11c+CD123+, demonstrated the same results for IMT patients. Particular T cells subsets (T CD8, activated HLA-DR+CD38+ CD8 T, memory CCR7-CD45RA+ CD8 T and Treg cells) were found significantly increased in IMT patients. Preliminary results suggest in all NSCLC patients a significant decrease in the ratios of effector: suppressor cells (E: S ratio), considering MDSCs and Tregs as suppressors and CD8 T cells and NK cells as effectors. In IMT NSCLC patients, a significant decrease in PD-1-expressing cells, and in some cases, decrease of density of PD-1 was observed for CD4 T (including Treg cells) and CD8 T cells, monocytes and dendritic cells. In conclusion, alterations found in the analyzed subsets in IMT NSCLC patients could be important candidates for evaluation of response in immunotherapy in clinical practice. Further studies are necessary to validate data.
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No conflict of interest

J2.

Selected for oral presentation – Symposium V

J3. Hereditary and sporadic neoplastic lesions of the colon show a distinct immune profile

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Introduction: Tumor-infiltrating immune cells are key players in the tumor microenvironment. T regulatory cells (Tregs) infiltrate tumors and induce a tumor-protective immune tolerance. It is known that peripheral Treg conversion seldom occurs in cancer and that Tregs present in tumors are mainly generated in the thymus, which undergoes a process of involution throughout life. These arguments make us believe that Treg-mediated immune subversion occurs in a different way in early and late onset cancers. We hypothesize that in hereditary cancers, tolerance is achieved by negative selection of tumor-reactive T lymphocytes. On the other hand, in sporadic cancers, positive selection of suppressor Tregs may be involved in the generation of tumor tolerance. To test this hypothesis, the immune infiltrate in neoplastic lesions of patients with familial adenomatous polyposis (FAP) and sporadic colorectal cancer (CRC) was analyzed by immunohistochemistry for CD3, CD4, CD8, CD57, CD68 and FoxP3 markers. We first analyzed a group of 14 patients (9 FAP + 5 sporadic CRC) with early and late stage neoplastic lesions of the colon (low grade dysplasia (LGD), high grade dysplasia (HGD) and adenocarcinoma (ADC)). The analysis of a cohort of 51 patients with LGD lesions (21 FAP + 30 sporadic CRC) is ongoing. We observed that the immune response to developing tumors was distinct between sporadic and hereditary backgrounds. More specifically, sporadic CRC cases tended to present higher infiltration with immune cells. Regarding the first group of patients, we observed higher densities of FoxP3+ cells in sporadic CRC lesions from all types (p(LGD)=0.013, p(HGD)=0.019, p(ADC)=0.034) when compared with FAP lesions. The results concerning ongoing work have so far corroborated these data. These observations are in line with the hypothesis initially raised, and they support the role of Tregs in the development of a central tolerance component in the process of tumor escape to immunosurveillance.

No conflict of interest

J4. Multivalent PLGA-based nanoparticles for combinatorial innovative colorectal cancer immunotherapy and immunomodulation

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Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth cause of cancer death worldwide. It is respossible for approximately 700,000 deaths per year. This work focused on the development of a combinatorial multivalent nanoplatform for CRC immunotherapy and immunomodulation based on the design of polymeric nanoparticles (NP) able to deliver a combination of CRC-associated antigen, adjuvants and gene regulators according to targeted cells, dendritic cell (DC) and CRC cells. Poly(lactic-co-glycolic) (PLGA)-based NP were prepared by the double emulsion (w/o/w) solvent evaporation method. NP were physicochemically characterized in terms of size, zeta potential and surface morphology. CRC antigen loadings were quantified by fluorescence. Immature DC (ATCC® CRL-11904TM) were used to evaluate the in vitro NP cytotoxicity by the propidium iodide assay, as well as NP cellular uptake profile by flow cytometry. In vivo biodistribution assay of plain NP was also performed using the IVIS Lumina® Bioimaging system. NP uptake in vivo by myeloid antigen presenting cells and the expression of maturation and co-stimulatory molecules at the surface of these cells sorted within draining lymph nodes, were also evaluated by flow cytometry. PLGA-based NP presented a mean size diameter close to 200 nm, with low polydispersity index (PdI) (≤0.200), a surface charge close to neutrality, as well as, a spherical shape and smooth surface. These multivalent delivery systems presented high loadings for antigen and adjuvants. No cytotoxic effect was observed on immature DC up to 48 h of incubation. NP were extensively internalized by immature DC in vitro after 48 h incubation, and by migratory DC in vivo 17 h after animal immunization. In vivo real-time monitoring of NP accumulation in mice whole bodies and dissected organs showed a fluorescent signal at 17 h close to the site of immunization and in the lymph nodes. No significant differences in the expression of the co-stimulatory CD80, CD86 and MHC class I markers on CD11b+CD11c+MHCII+ population at lymph nodes were observed among different polymeric

combinations upon mice immunization with NP carrying CRC antigen and adjuvant. According to NP physicochemical characteristics, internalization and biodistribution patterns, this innovative nanoplatform can lead to a safe multivalent nanomedicine able to modulate dendritic cell activity and T cell expansion against tumor cells expressing entrapped antigens.

No conflict of interest

J5. New small-molecule immune system modulators: A therapeutic alternative to monoclonal antibodies

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Introduction: Immunotherapy is currently a powerful strategy in cancer therapy with very exciting outcomes. In particular, modulation of immune checkpoint receptors have gain special attention. These immune regulators limit proliferation and activity of T cells and other immune cells enrolled in these signaling pathways. Under normal conditions, they are essential in modulation of immune responses; however, they are also one of the major mechanisms used by tumors to evade immune system recognition and destruction. To date, several immune checkpoint receptors have been identified and used as therapeutics in oncology, as programmed cell death protein 1 (PD-1). When engaged by one of its ligands, PD ligand 1 (PD-L1) or PD ligand 2, PD-1 limits autoimmunity. PD-1 ligands are upregulated in many human cancers and their blockade lead to activation of T cells and therefore enforce tumor recognition. Presently, PD-1/PD-L1 pathway is one of the most successful pathways in the context of clinical cancer immunotherapy with several approved drugs. The most successful therapies relay on the use of antibodies. However, despite their outstanding success, they still have numerous disadvantages. Small-molecule modulators have emerged as safer therapeutic alternative. Our study focused the discovery of small-molecule inhibitors targeting PD-L1 to disrupt PD-1/PD-L1 interaction. Limited structural information of PD-L1 led us to a detailed structural characterization based on in silico studies. After assessing structural features (e.g. binding pocket, flexibility or gating), we followed a structure based virtual screening campaign. Potential PD-L1 inhibitors were selected based on small-molecule affinity to PD-L1. Virtual screening campaign was validated using Homogeneous Time Resolved Fluorescence (HTRF) assay to assess compound activity. So far, we were able to identify small-molecule PD-L1 inhibitors that will be tested in vitro. Therefore, immune checkpoint blockade using small molecules represent a step forward in cancer immunotherapy.

No conflict of interest

K1. Characterization of FaDu cell line and evaluation of ionizing radiation effect - in vitro preliminary results

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Introduction: Head and neck cancer is the sixth leading cancer worldwide. It is an heterogeneous group of malignancies that commonly arise in upper aerodigestive tract mucosa, including hypopharyngeal tumour. Despite this cancer has a low incidence, patients have disease advanced stage with poor prognosis and distant metastasis. The cancer stem cells subpopulation (CSC) is suggested as responsible for low overall survival. Identification of a CSCs markers panel could help to overcome this barrier allowing to find potential treatment targets. The aim of this study is evaluate and characterize the expression of proliferation, adhesion and CSC markers in a hypopharyngeal cell line (FaDu) as well as the effect of ionizing radiation exposure. Materials and Methods: The pattern of expression was evaluated by immunohistochemistry (IHC) across paraffin cell blocks of control cells. Therefore, was performed epithelial characterization (cytokeratin CAM 5.2, leukocyte common antigen (LCA), vimentin), tumoral aggressiveness (P16, P53, OCT4 and SALL4), proliferation (Ki-67) and CSC markers (EpCAM, CD10, CD44, CD117, CD133, beta-catenin). Also, 14 subtypes of high-risk HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 16, and 18), EBV, Akt and Wnt expressions were analyzed. FaDu cells in a concentration of 0.5×10^6 cells/mL were exposed to increasing doses of X-ray (0.5 to 10 Gy), with exception of control cell, followed by clonogenic assay and IHC. For IHC, 48h after treatment, cells were fixed with alcohol 96% and posteriorly were analyzed the following antigens Ki-67, EpCAM, CD133, CD44, beta-catenin, CD117, CD10, Akt, Wnt, OCT4 and P53. Results and Discussion: Relatively to epithelial characterization, FaDu expressed cytokeratin+, vimentin+ and LCA-. About tumoral aggressiveness and proliferation, cells only expressed positively P16 and was Ki-67+. Stemness was characterized by positive expression of CD44, CD133 and beta-catenin. Between HPV subtypes analyzed, FaDu was positive to HPV18 and negative to EBV. Moreover, control cells expressed Akt+ and Wnt+ in normal conditions. Preliminary results demonstrated that cell survival is ionizing radiation dose dependent. In addition, it was observed Wnt expression alterations with X-ray exposure. These results suggest that this molecular pattern can be related to FaDu survival, once this molecule is involved in cell fate determination, cell polarity, cell migration and cell proliferation.

No conflict of interest

K2. Esophageal cancer therapy response: role of hypoxia in epigenetic mechanism regulation

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Introduction: Esophageal cancer, comprising mostly two major histological subtypes - squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) - is the eighth most common cancer and the sixth leading cause of death worldwide. Despite the advances in radiotherapy, esophageal cancer prognosis remains poor. Hypoxic foci in solid tumors are known to endure resistance to radiotherapy. At molecular level, hypoxic microenvironment is characterized by increased transcription and activation of hypoxia inducible factors' (HIF). This was suggested to associate with aberrant post-transcriptional modifications and chromatin remodelers' deregulation, leading to inappropriate gene activation or repression. However, the interplay between hypoxia and epigenetics is not fully understood. Hence, our aim is to tackle the association between epigenetic factors, hypoxia and radiation therapy. Importantly, we intend to investigate whether those epigenetic changes may be used as predictive markers for esophageal cancer response to radiotherapy under conditions of hypoxia. Materials and Methods: ESCC cell lines were irradiated with RT dosages ranging from 0 to 8Gy, in presence of 50uM CoCl2 and the effect on cell survival was assessed through colony formation assay. The expression of different epigenetic markers and hypoxia factor (HIF-1 alpha) were assessed at transcriptional and protein level, in the presence of CoCl2 treatment, before and after radiotherapy, through RT-qPCR, Western blot and immunofluorescence assays. Results and Discussion: Increased HIF-1 alpha expression was observed in CoCl2 exposed cells compared to basal conditions. Moreover, significantly increased transcriptional levels of histone demethylases (KDMs-erasers), namely KDM3A, KDM4C and KDM6B, were depicted for all studied cell lines, paralleled at protein levels by decreased H3K9me3 and H3K27me3 expression (targeted by KDMs). Similar findings were observed in cell lines exposed to radiotherapy. Thus, our results suggest that that these histone demethylases may be predictive biomarkers of response to radiotherapy and their inhibition may increase radiosensitivity in esophageal cancer.

No conflict of interest

No abstracts

Topic M
PREVENTION AND EARLY DETECTION

M1. Nanoparticle-Enhanced Molecular Fluorescence-Endoscopy for detection of early-stage colorectal adenocarcinomas

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Introduction: Colorectal cancer affects approximately 6% of the population and is the second leading cause of cancer-related death in the US and Europe, mainly because the disease is not detected early enough. Colonoscopy is the only technique that allows recognition and immediate therapy of precursor changes in the colon wall and it is recommended for all people at a certain age depending on their colorectal cancer-risk. However, early stage lesions can occur in the mucosal epithelium without causing any morphological changes visible at colonoscopy. To improve the detection of early-stage colorectal cancer, nanoparticle-enhanced fluorescence endoscopy was proposed in this study. The concept is to develop gold nanoparticles conjugated with antibodies directed specifically to heavily up-regulated molecules on tumour cells, and thus to enhance diagnosis by colonoscopy. Materials and Methods: Gold nanoparticles were synthesised by the citrate reduction method (Turkevitch method) and functionalized with a tripeptide. The gold nanoparticles were conjugated to anti-EPCAM antibody via thiolation of the antibody. The nanoconjugates were characterized by using UV-VIS, dynamic light scattering and laser doppler velocimetry, attenuated total reflectance-Fourier Transform infrared spectroscopy, transmission electron microscopy techniques, in vitro drug stability analysis. Darkfield macroscopy assay was performed to reveal tumour imaging on human colon carcinoma sections with the incubation with conjugated gold nanoparticles with anti-EpCAM antibody. Results and Discussion: A stable and well-defined nanosystem based spherical functionalized gold nanoparticles was successfully designed and prepared, then conjugated them with Chlorin e6. The conjugation efficiency of chorin e6 on gold nanoparticles determined was 94% corresponding to 18.8 M of Ce6 on the nanoparticles. Gold nanoparticles were conjugated with the anti-EpCAM antibody, producing stable nanoparticles with mean diameter of 39 nm and a zeta potential of -22 mV. The nanoconjugates were taken up by the colon carcinoma cell line MC38 and recognized their fluorescence inside the cells. The gold nanoparticles were visualized by either fluorescence or by reflectance imaging, both available in clinical endoscopy. The nanoconjugates developed validated successfully the proof of this study concept, that early colon adenocarcinomas are detectable by topical application of tagged targeted nanoparticles.

No conflict of interest

M2. Can we eat to prevent colorectal cancer? The role of Mediterranean diet

Pires A.S.[1], Roxo R.[1], Abrantes A.M.[1], Guilherme C.[2], Neves A.R.[1], Pereira C.[3], Oliveira E.[4], Cristina I.[5], Tralhão J.G.[6,7], Botelho M.F.[1]

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Introduction: Industrialization, western lifestyle and changes in environmental and dietary factors are possible causes of the increasing prevalence of colorectal cancer (CRC). Mediterranean diet (MDiet) is considered one of the healthiest diet models because it has positive functional effects on the health and well-being. Several studies suggest that the adherence to MDiet prevents the development of certain types of cancer, due to the role of dietary fiber, as well as, the diversity of vitamins and substances with antioxidant properties. To date, there are few studies conducted in the Portuguese population. This work constitutes an exploratory study in the "Região Centro" of Portugal and aims to understand the impact of diet on CRC. Methods: 266 subjects (164 control, 102 CRC cases) were asked to sign a proper informed consent and answer a Food Frequency Questionnaire validated for Portuguese population, to evaluate their eating habits. The conversion of food into nutrients was done using Food Processor Plus. Nutritional information allowed the determination of the adherence to MDiet through the MAI (Mediterranean Adequacy Index). Anthropometric and lifestyle data were collected and physical exercise was quantified by the consumption of kilocalories per week (kcal/w). Risk factors of CRC were analyzed. Data obtained allowed to compare several parameters between control and cases groups, using SPSS. The project was approved by 4 Ethics Committees. Results and discussion: CRC group showed a statistically significant increase in calories, proteins, carbohydrates, fat, sugar and cholesterol intake compared to the control group ($p<0.001$). In contrast, the control group had a consumption of kcal/w significantly higher than CRC group ($p<0.001$). Studied population showed a low adherence to MDiet, not allowing obtaining differences between groups. Regarding CRC risk factors, CRC individuals are more constipated ($p<0.001$) and have higher body mass index values (excess weight). Multivariate analysis, using a logistic regression model ($\chi2=40,3$; $p<0,001$), demonstrated that individuals are more likely to develop CRC if they are constipated and stay more hours without eating in between meals. Diet of CRC patients was characterized by the generalized high consumption of nutrients. Physical exercise was associated with a lower risk of CRC. Additional studies may promote the establishment of dietary guidelines adapted to the Portuguese population for CRC prevention.

No conflict of interest

M3. Early detection of the three major primary cancers in women by cell-free DNA methylation in liquid biopsies

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Introduction: Breast, colorectal and lung cancers are three most common and deadly cancers in females in developed countries. Advances in cancer screening led to an increase in early stage disease detection, especially in breast cancer. However, screening tests might be hampered by high false-positive rate and overdiagnosis, and despite their implementation cancer-related mortality remains high. Thus, new screening approaches are urgently needed for these three major cancers. Epigenetic alterations, especially aberrant DNA methylation, play an important role in cancer development. Because DNA methylation is an early event in cancer progression and can be detected in circulating cell free DNA (ccfDNA), it has been proposed as a valuable cancer biomarker. Therefore, the assessment of DNA methylation in a liquid biopsy represents a potential non-invasive test for early cancer detection. The major aim of this study was to develop a sensitive and specific methylation-based test in liquid biopsies' cell-free DNA for detection of the three most incident cancers in women. Material and Methods: CcfDNA was extracted from plasma of breast, lung and colorectal

cancer patients and healthy donors. Next, ccfDNA was subjected to sodium-bisulfite modification and whole-genome amplification. APC, RASSF1A, and FOXA1 promoter methylation levels were then assessed by multiplex quantitative methylation-specific PCR. ROC curves were constructed to assess the diagnostic performance of the tested biomarkers. Results and discussion: APC, RASSF1A and FOXA1 hypermethylation was found in plasma samples from breast, lung and colorectal cancer patients. The best performance was displayed by FOXA1 promoter methylation. Moreover, the panel APC, RASSF1A and FOXA1 showed a good performance in detecting all three cancers, with sensitivity and specificity higher than 70%. Thus, DNA methylation-based biomarkers might be valuable for early detection of the three major cancers in women, using a minimally-invasive strategy.

No conflict of interest

N1. R68G as putative marker associated with antiproliferative effect on a breast cancer cell model

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Introduction: Breast cancer is one of the most frequent cause of female cancer death (1). Triple-negative breast cancer (TNBC), currently does not have a specific target treatment, and the chemotherapy approaches frequently used are not completely efficient. Therefore, it is important to develop new TNBC therapeutic strategies, as preventive or complementary approaches. Natural bioactive compounds have been the basis of several new pharmaceutical products. Cynara cardunculus L. (Cc) is well known for its therapeutic activities, and its use in folk medicine since ancient times. Cc represents a natural source of terpenic compounds, with the predominant molecule being cynaropicrin (Cyn), a sesquiterpene lactone, with recognized biological properties, including anti-tumor (3). The germacrene A synthase gene (GAS) is involved in the initial step of Cyn biosynthesis, and GAS expression levels showed a correlation with Cyn content (4). The present work aimed to identify new allelic variants in GAS gene involved in Cyn biosynthesis, and correlate them with improved biological activities, such as the antiproliferative effect of Cc leaves-derived lipophilic extracts (CLE), and Cyn, using the human TNBC MDA-MB-231 cell line, as model. Using high resolution melting, 9 haplotypes were identified and the putative impact of the identified allelic variants in GAS was evaluated by bioinformatics tools. Cell viability was determined by MTT assay; flow cytometry for cell cycle analysis and western blotting for AKT molecular signalling and cell cycle markers were performed. In this work, we identified, in a Cc population, one allelic variant R68G in GAS gene sequence with significant association with MDA-MB-231 cells antiproliferative activity. In fact, cultivated cardoon leaves lipophilic extract (IC50 10.39 µg/mL) and cynaropicrin (IC50 6.19 µg/mL) could prevent MDA-MB-231 cell growth, with significant relative protein expression level increments of important G2/mitosis checkpoint proteins, namely p21Waf1/Cip1, p-Try15-CDK1 and cyclin B1, probably related to G2 cell cycle arrest. Moreover, cynaropicrin and CLE decreased significantly the relative protein expression of p-Ser473-Akt in MDA-MB-231 cells. In sum, we identified the allelic variant R68G in GAS sequence, being associated with the tumor antiproliferative action of CLE, which can prospective Cc plantations with a valuable bioactive composition, mainly of cynaropicrin, toward a natural-based TNBC therapeutic approach.

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N2. microRNAs in oncology. Aplicability in pancreatic cancer

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Introduction: Malignant neoplasms are a real worldwide threat to public health, being the second cause of death. The overall burden of cancer is rising fast and it is expected to continue to increase in the coming decades. Pancreatic cancer, although not the most prevalent cancer, being only the 11th most widespread worldwide, it is the seventh cause of death by cancer, for both genders. Patients diagnosed with pancreatic cancer have a high mortality rate, roughly the same number of diagnosed cases, hence its lethal nature. The increase in cancer incidence worldwide fosters new challenges and the need to develop technologies to help fight this burden. Among them, biomarkers that can help an early identification of cancer, monitorization, therapy and aid in prognostics are promising future solutions. miRNA's are small non-coding sequences of RNA that regulate gene expression. Given their important function, it is expected that their deregulation is involved in various pathologic processes, namely cancer. These small molecules have been extensively researched as biomarkers. On pancreatic cancer, tumor circulating miRNA's have been identified, usually over or underregulated, and have been characterized as diagnostic or prognostic biomarkers. miRNA-21, miRNA-210 and miRNA-155 are some of the identified sequences in pancreatic cancer. Pancreatic cancer has a high metastization rate, and the identification of miRNA's like 10b, 21, 31 and others, which are related to metastization phenomena, strengthens research on miRNA's to further understand the evolution and behaviour of pancreatic cancer, and ultimately to be used as a therapeutic agent.

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N3. Solid Lipid Nanoparticles for Glioblastoma Multiforme therapy

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Introduction: Solid lipid nanoparticles (SLN) have been studied as an efficient drug delivery system due to its well established clinical safety and the ability to enhance drug therapeutic benefits [1]. In this work, our approach is based in SLN for temozolomide (TMZ) delivery, an alkylating agent used for glioblastoma multiforme (GBM) treatment. The classical TMZ therapy is rarely curative due to this type of tumour heterogeneity's, anatomic location and high proliferation rate [2]. TMZ exhibits some limitations as high toxicity and low availability to target the cancer tissues, as many other chemotherapeutic drugs. To overcome these imitations, TMZ was encapsulated in SLN. Materials and methods: Cetyl palmitate-based SLN were prepared using the hot homogenization, high-shear and ultrasound dispersing techniques. The NPs were stabilized with poloxamer 407. The developed nanocarriers were characterized in terms of size, size distribution and zeta potential using the dynamic light scattering technique. Results and discussion: The attained SLN showed mean diameters of about 185 nm and encapsulation efficiency of 35%. The produced nanocarriers also exhibited negative zeta potential values and stored at room temperature are stable for at least one month. In future work, a targeting approach will be used with SLN modified with anti-transferrin receptor (TfR) monoclonal antibody (mAb), since this receptor is overexpressed in GBM cells and in the blood brain barrier [3]. Our final objective is to evaluate the antiproliferative effect of TMZ entrapped in the SLN on human GBM cell lines and compare cytotoxicity efficacy with free TMZ. Acknowledgments: This work was the result of the project POCI-01-0145-FEDER-006939 (LEPABE – UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; and

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N4. The expression of breast cancer stem cell markers in brain metastasis is associated with disease aggressiveness

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Introduction: Breast cancer brain metastases represent a major clinical challenge with very limited therapeutic approaches. Around 40% of human epidermal growth factor receptor (HER) 2-positive and triple-negative breast cancer patients are at higher risk of developing brain metastasis. Since breast cancer stem cells (BCSCs) were described as being responsible for metastases initiation, the aim of this study was to characterize the expression of BCSC markers in a brain-seeking (BR) breast cancer cell model derived from the triple-negative MDA-MB-231 cell line and in human brain metastases. Material and Methods: Western-blot and flow cytometry were used to evaluate the expression of BCSC markers and signaling in BR cell lines. The chick embryo chorioallantoic membrane (CAM) assay was used as a model to assess the tumorigenic potential of these cells. Finally, BCSC markers were evaluated in 29 human brain metastases by immunohistochemistry and associated with patient survival. Results and discussion: We found that BR cells exhibit higher expression of the BCSC markers P-cadherin, CD44, CD24, CD49f and EpCam in comparison with parental cells. Src/FAK signaling was also significantly increased in BR cells. A preliminary CAM assay showed that tumors formed by BR cells are larger than those of the parental cell line. In addition, we found that P-cadherin expression in breast cancer brain metastasis was associated with worse progression free survival, whereas CD44 and EpCam were associated with worse brain metastasis free survival. Although still preliminary, our data suggests that breast cancer cells showing stem cell features are associated with characteristics that promote a higher metastatic potential and progression of the disease. Further experiments will be performed, namely CAM assays to study the ability of the BR cells to form metastasis. Mammosphere and ALDEFLUOR assays will also be used to assess stem capacity of BR cells and to study the functional impact when manipulating these BCSC markers.

No conflict of interest

N5. Annotator: a novel custom tool for genomic variants annotation and classification

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Introduction: Genome sequencing produces large amounts of data that enable the discovery of genomic variants. Using appropriate bioinformatics tools and public databases which aggregate clinical information, it is

possible to understand the functional impact of such variants on human phenotypic traits. Several bioinformatics tools already exist, however with limitations such as, the license costs and the limited choice of public databases consulted. Therefore, our aim was to implement an in-house tool that aggregates information collected from selected public databases, allowing the custom annotation of genomic variants and their classification in terms of pathogenicity. Materials and Methods: We implemented a tool, Annotator, which annotates and classifies genomic variants using data from 5 renowned public databases (Uniprot, OMIM, ClinVar, dbSNP, Pubmed). Annotator can also integrate keywords, defined according to sample characteristics, for advanced text mining, in order to further select collected data to best suit the clinical features of the sample under analysis. Results and Discussion: Annotator was used to analyze and classify a set of 151 genomic variants, detected in 52 probands with Familial Intestinal Gastric Cancer. These genomic variants had already been analyzed/classified using a commercial software. The comparison of the results obtained with both analyses showed that Annotator was able to collect further information for 7/42 somatic variants and 4/24 germline variants, which were classified as unknown significance for the commercial software. Annotator is a valid tool for an accurate annotation and efficient classification of genomic variants derived from sequencing experiments.

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