2ND ASPIC INTERNATIONAL CONGRESS

PROCEEDINGS BOOK

IPO-PORTO

28-29 APRIL 2016

ASPIC
ASSOCIAÇÃO PORTUGUESA DE INVESTIGAÇÃO EM CANCRO

LETTER OF WELCOME

On behalf of the ASPIC Board of Directors and of the Organizing and Scientific Committee of the 2nd ASPIC International Congress, it is our pleasure to invite you to join the 2nd ASPIC International Congress to be held on April 28-29, 2016, at the IPO-Porto Auditorium, in Porto, Portugal.

The 2nd ASPIC Congress is part of the Porto Cancer Meeting organized by Ipatimup for more than 20 years and also of the Porto Comprehensive Cancer Centre and thus highlights the engagement of Porto in the national cancer research initiatives.

The upcoming 2nd ASPIC International Congress will be an excellent opportunity to share knowledge on the new developments at basic and clinical levels in cancer immunology, tumor biology, signaling pathways and radiobiology.

We expect that the scientific community will take an active part in this conference, benefit from the exciting scientific programme and take the opportunity to make new contacts and establish new collaborations. Young scientists will have the time and space to meet experts in different fields. It is our aim to create an open and engaging environment for discussion and to liaise portuguese scientists with basic background with clinically oriented professionals.

Porto is a perfect venue for a scientific congress. We have excellent cancer research centers in a city living an especially stimulating moment. The historic center of the city was listed as a UNESCO World Heritage, and the different kinds of architectural styles, exquisite gastronomy, the Douro river, the Port Wine cellars, art galleries and charming streets with a still unique environment, are a perfect setting for the event.

We look forward to welcoming you in April 2016.

José Laranja Pontes　　　　　　　　*Manuel Sobrinho Simões*
Congress President　　　　　　　　　*Congress Vice-president*

CONTENTS

ASPIC

ASPIC, the Portuguese Association for Cancer Research, was created in 2013 and promotes cancer research in all its aspects and in public benefit. The association encourages excellence, disseminates results, analyzes and proposes solutions for relevant questions for cancer research and cancer investigators in Portugal and at the international level taking advantage of its affiliation with the european partner – EACR.

Membership benefits include:

- Automatic affiliation to the European Association for Cancer Research
- Reduced registration rates at meetings organized by ASPIC, ASEICA, EACR and ECCO
- Access to scholarships and awards
- Contacts and opportunities for collaborative research
- Reduced subscription rates to the European Journal of Cancer

Membership fees:

- Active Member   40€/year, 120€/4 years
- Post-doc students   20€/year, 60€/4 years
- Master or PhD students   25€/4 years

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ACCREDITATION INFORMATION

The 2nd ASPIC International Congress is accredited by the **European Accreditation for Continuing Medical Education** (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The 2nd ASPIC International Congress is designated for a maximum of 11 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full–day event.

European Accreditation is granted by the EACCME in order to allow participants who attended the above-mentioned activity to validate their credits in their own country.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credits to AMA credits can be found at: www.ama-assn.org/go/internationalcme.

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

CONGRESS PROGRAMME

THURSDAY, 28 APRIL

09.00 **Opening Session**

Symposium I
TUMOUR IMMUNOLOGY
Chairs: José Dinis, Jocelyne Demengeot

INVITED SPEAKERS

09.30 Bruno Silva Santos . Portugal
Gamma-delta T lymphocytes in the tumour environment: from oncoimmunology to adoptive cell immunotherapy.

10.00 Jolanda de Vries . Netherlands
Harnessing adaptive immune responses in hereditary cancer.

SELECTED SPEAKERS

10.30 Célia Gomes . Portugal
Natural Killer cell-based adoptive immunotherapy eradicates and promotes the differentiation of chemoresistant stem-like cells.

10.45 Karine Serre . Portugal
Tumour-associated neutrophils inhibit pro-tumour IL17-producing gamma-delta T cells.

11.00 Coffee-break

Symposium II
IMMUNOMODULATION OF CANCER
Chairs: Bruno Silva Santos, Júlio Oliveira

INVITED SPEAKERS

11.30 Frederick Arce Vargas . United Kingdom
Targeting immune regulation in the tumour site.

12.00 Jeffrey Weber . United States
Efficacy and Biomarker Analyses of the Use of Checkpoint Inhibition in Melanoma.

SELECTED SPEAKERS

12.30 Ana Catarina Pinho . Portugal
Functional capacity of tumor lysate-pulsed monocyte-derived dendritic cells inducing in vitro immune responses

12.30 Paulo Rodrigues-Santos . Portugal
PD-1 downregulation on Tregs during Tyrosine Kinase Inhibitor therapy in Chronic Myeloid Leukemia

13.00 Lunch

14.00 **Poster Parallel Sessions**
Chairs: A.T. Maia, A. Preto, F. Carneiro, G. Sousa, I. Fonseca, M. Damasceno, M.J. Cardoso, M.J. Bento, N. Lunet, N. Sousa, R. Seruca, R. Henrique, S. Socorro

16.00 Coffee-break

Symposium III
RADIATION BIOLOGY AND RADIOTHERAPY
Chairs: Luísa Carvalho, Pedro Almeida

INVITED SPEAKERS

16.30 Rolf Lewensohn . Sweden
Molecular tumour response to accelerated ions and role of cellular modifying factors.

17.00 Marie Dutreix . France
Focus on the combination of DNA repair inhibitors with radiotherapy.

SELECTED SPEAKERS

17.30 Paula Boaventura . Portugal
TERT promoter mutations in thyroid carcinoma from childhood irradiated tinea capitis patients.

17.45 Ana T. Pinto . Portugal
Macrophages modulate colorectal cancer cell response to radiation.

18.00
19.00 **ASPIC General Assembly**

20.00 Congress Dinner

FRIDAY, 29 APRIL

Symposium IV
SIGNALING PATHWAYS: THE ANDROGEN RECEPTOR MODEL

INVITED SPEAKERS

09.30   Wytske van Weerden . Netherlands
The continued role of AR in castration-resistant prostate cancer.

10.00   Tiffany Traina . United States
The role of the AR in advanced breast cancer.

SELECTED SPEAKERS

10.30   Cristina Amaral . Portugal
Estrogen-dependent breast cancer: the involvement of androgen receptor in exemestane-acquired resistance.

10.45   Inês Graça . Portugal
Androgen Receptor mediates castration-resistant prostate cancer cells response to the DNMT inhibitor Hydralazine.

11.00   Coffee-break

Symposium V
CELL AND TUMOUR BIOLOGY
Chairs: Luís Costa, Fátima Baltazar

INVITED SPEAKERS

11.30   Gunther Boysen . United Kingdom
Molecular features of SPOP mutant/ CHD1 deleted prostate cancer.

12.00   Joan Seoane . Spain
Tackling intratumour heterogeneity in brain tumors.

SELECTED SPEAKERS

12.30   Joana Vieira de Castro . Portugal
Autofluorescence as a new biomarker to identify Glioblastoma Stem Cells.

12.45   Ana Sofia Ribeiro . Portugal
P-cadherin, a therapy predictive biomarker for Dasatinib in basal-like triple negative breast cancer.

13.00   Lunch

PLENARY LECTURES
Chairs: Manuel Sobrinho Simões, Nuno Miranda, Jorge Soares, Carlos Camps

ASPIC Lecture

14.00   Alberto Cambrosio . Canada
Mapping the development of Portuguese clinical research in oncology.

ASEICA Lecture

14.45   Luis Paz Ares . Spain
Lung cancer precision treatment.

EACR Lecture

15.30   Richard Marais . United Kingdom
RAS and RAF signalling in melanoma: from basic biology to clinical responses.

16.15   **Closing Session**

Research Trial / Pixels Brand Oral Presentation Prize

EACR Poster Prizes

OLI Poster Prizes

ABSTRACTS

INVITED SPEAKER

SI 1. Gamma-delta T lymphocytes in the tumour environment: from oncoimmunology to adoptive cell immunotherapy.

Bruno Silva Santos

γδ T lymphocytes are important anti-tumour effectors due to their potent cytotoxicity and interferon-γ (IFN-γ) production, which underlie their non-redundant protective functions in vivo (Silva-Santos et al. Nat Rev Immunol 2015). Strikingly, a recent analysis of a collection of 39 cancer types revealed intratumoural γδ T cells as the most significant favourable prognostic immune population (Gentles et al. Nat Med 2015). This notwithstanding, we were among the first to describe an unanticipated tumour-promoting role for murine γδ T cells in an ovarian cancer model, which was linked to the production of interleukin-17 (IL-17) and the mobilization of pro-tumour macrophages that enhanced angiogenesis and tumour cell proliferation (Rei et al. Proc Nat Acad Sci USA 2014). Thus, the γδ T cell response is pleiotropic (Rei et al. Cancer Res 2015) and requires selective modulation for therapeutic benefit. Aiming for therapeutic translation of our research, we devised a clinical-grade cellular product composed of in vitro activated and expanded human γδ T-cells that selectively recognize malignant (but not normal) leukocytes, and produce IFN-γ (and TNF) and but no IL-17. This subset expresses the Vdelta1 TCR and a broad repertoire of natural killer receptors, including NKG2D and the natural cytotoxicity receptors (NCRs), NKp30, NKp44 and NKp46. Critically, NCR expression is absent in freshly-isolated γδ T-cells but is selectively induced on Vdelta1 T-cells by a cocktail of cytokines and TCR agonists. NCR expression endows these lymphocytes, termed Delta One T (DOT-) cells®, with enhanced cytotoxicity against lymphoid and myeloid leukemia cells in vitro. Importantly, DOT-Cells® were able to target and eliminate chemoresistant acute myeloid leukemia cells. We further employed xenograft models of human chronic lymphocytic leukemia in NOD-SCID c-/- (NSG) hosts to show that DOT-Cells® infiltrated the tumour and various other tissues, and were strikingly capable of preventing tumour dissemination to organs such as bone marrow and liver. No evidence of treatment-associated toxicity was found in biochemical (blood) or histological (multiple organs) analyses. These data provide the proof-of-concept for pioneering the adoptive transfer of DOT-Cells® in cancer clinical trials.

Bruno Silva-Santos is a Principal Investigator and Vice-Director of Instituto de Medicina Molecular (IMM), and Associate Professor (with Habilitation) of the Faculty of Medicine of the University of Lisbon, Portugal. He did his PhD (1998-2002) with Dr. Mike Owen at The London Research Institute (CRUK), and trained as a post-doc (2002-2005) with Prof. Adrian Hayday at King's College London. He returned to Portugal in 2006 to establish a molecular immunology laboratory at IMM. His research is dedicated to T lymphocytes and their key roles in immunity to infection and cancer. His projects range from the development of these cells in the thymus, to their functions upon infection or tumour challenge. The European Research Council (ERC) awarded him a Starting Grant in 2010 and a Consolidator Grant in 2015. He was also selected to the EMBO Young Investigator Programme (in 2011) and nominated to the European Academy of Tumour Immunology (in 2012). He serves as editor for four peer-reviewed journals and heads the Scientific Council of Lymphact SA, a biotech company focused on cancer immunotherapy.

SI 2. Harnessing adaptive immune responses in hereditary cancer.

Jolanda de Vries

(no abstract available)

Jolanda de Vries is a Professor at the Department of Tumor Immunology at the Nijmegen Centre for Molecular Life Sciences. She was one of the pioneers to translate dendritic cell biology into potential clinical applications. The first clinical phase I/II studies in which patients were vaccinated with DCs loaded with tumor-specific peptides were initiated in 1997. She also developed a novel immuno-monitoring assay that is highly predictive for extended survival after vaccination with DCs (J Clin Oncology 2005). Her primary scientific interest continues along the line of DC-immunotherapy and in particular the migration and imaging of DC. For example, in-vivo imaging of ex-vivo labeled cells using MRI (Nature Biotechnology 2005). New opportunities for other cell-types (e.g. subsets of DCs) and combination therapies are now being developed.

SI 3. Natural Killer cell-based adoptive immunotherapy eradicates and promotes the differentiation of chemoresistant stem-like cells

Ferreira-Teixeira M.[1,2], Paiva-Oliveira D.[1,2], Parada B.[1,3], Alves V.[4], Chijioke O.[5] , Münz C.[5] , Reis F.[1,2], Rodrigues-Santos P.[4,6], Gomes CM.[1,2]

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Introduction: Bladder Cancer (BC) is the fifth most common cancer in western world, currently treated by transurethal resection and intravesical mitomycin or BCG in non-muscle invasive forms. This tumor has a high propensity for recurrences and progression that appears to be related to the presence of Cancer Stem Cells (CSC) that are refractory to standard therapies. In this study we evaluated the therapeutic potential of allogeneic activated Natural Killer-cell based adoptive immunotherapy against bladder cancer stem cells (CSCs) using in vitro and in vivo models. Material and methods: CSCs were isolated from two invasive BC cell lines (HT1376 and UMUC3) using the sphere-forming assay and analyzed for the expression of specific ligands that are recognized by activating and inhibitory receptors in NK cells. The cytolitic activity of IL-2/IL-15 activated NK cells isolated from healthy donors and BC patients against parental and CSC subsets was tested using either the CD107a degranulation or Cr-51 release assays. The antitumor activity of activated NK cells was evaluated in mice bearing a CSC-induced orthotopic BC. Results and discussion: NK cells from healthy donors and activated with IL-2 and IL-15 displayed an efficient anti-tumor activity against bladder cancer cells targeting both stem-like and differentiated bulk tumor cells. Moreover NK cell-mediated killing depended on expression of ligands for NKG2D and DNAM-1 activating receptors, and were partially abrogated by mAbs blockade. In addition to cell killing, NK cells shifted CSCs towards a more differentiated phenotype rendering them more susceptible to conventional chemotherapy. In opposite, NK cells from BC patients displayed low density of NCRs and of adhesion molecules and a more immature phenotype, and lost their ability to kill and drive differentiation of CSCs. The intravesical instillation of healthy activated-NK cells in mice resulted in a massive decrease in the tumor burden, ranging from 80% to complete remission after 4 treatments. Overall, the intravesical therapy with allogeneic activated-NK cells provides a rapid and noteworthy anti-tumoral response against BC by targeting both stem and non-stem cell populations and should be exploited as a complementary therapeutic strategy in BC patients. Funding: Astellas European Foundation Uro-Oncology Grant 2014, FCT SFRH/BD/77314/2011 and Strategic Project (PEst-C/SAU/UI3282/2011-2013 and UID/NEU/04539/2013) COMPETE-FEDER

No conflict of interest.

SI 4. Tumour-associated neutrophils inhibit pro-tumour IL17-producing gamma-delta T cells

Sofia Mensurado[1], Margarida Rei[2], Telma Lança[3], Hiroshi Kubo[1], Natacha Gonçalves-Sousa[1], Bruno Silva-Santos*[1] and Karine Serre*[1]

[1.]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal [2.]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine (or WIMM if you prefer to make it shorter) University of Oxford, UK [3]Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam

Introduction: The profile of tumour-associated immune cells holds great promise as biomarkers of clinical outcomes and potential immunotherapeutic targets. While tumour-infiltrating T cells associate with good prognosis, myeloid cells emerge as opposite predictors of survival for diverse solid tumours. Growing evidence shows that myeloid cells support tumour growth and metastasis by suppressing anti-tumour T cells and promoting angiogenesis. However, in some circumstances lymphoid subsets are subverted from their original task and promote tumour growth. Thus, the myeloid and lymphoid subsets, displaying potent anti- or pro-tumour effector functions, are actively "moving targets". And, while cancer progression results from a dynamic crosstalk between these myeloid and lymphoid effector cells, the exact nature this active dialogue remains to be elucidated. Materials and Methods We reported that in response to melanoma B16 cell line transplanted subcutaneously, gd T cells infiltrate the tumour, produce IFNg and efficiently delay tumour growth. By contrast, in response to ID8 ovarian cell line transplanted in the peritoneal cavity, gd T cells produce IL17 and attract inflammatory small peritoneal macrophages that directly support tumour growth. In the present work, we questioned whether a myeloid/lymphoid crosstalk regulates anti- versus pro-tumour functions of gd T cells. To determine the roles played by tumour type and/or location we injected B16 cells intraperitoneally. Results and Discussion We found that, in stark contrast with accumulating and IFNg-producing CD8 T cells, gd T cells failed to respond to melanoma B16 transplanted intraperitoneally. We hypothesized that, as already shown for conventional ab T cells, myeloid suppressive cells impact the gd T cell response. We observed a marked infiltration of neutrophils in the peritoneal cavity of B16-bearing mice. These cells were CD11b+Ly6G+Ly6Cint, displayed an immature phenotype and high inos gene expression. Critically, we depleted neutrophils, and while this restored gd T cell numbers they failed to produce IFNg. In fact, this treatment enhanced IL17A producing-gd T cells, a subset recently linked to tumour growth and metastasis formation. Thus, these data show for the first time, that neutrophils regulate the accumulation of IL17-producing gd T cells. This work underlines the importance of characterizing the pro-tumorigenic subsets, and crosstalk with lymphoid subsets, on a per tumour type basis.

No conflict of interest

ABSTRACTS

INVITED SPEAKER

SII 1. Targeting immune regulation in the tumour site

Frederick Arce Vargas

(no abstract available)

Frederick Arce Vargas trained as a surgical oncologist in the Universidad de Costa Rica. F.A.V. then joined Mary Collins group in University College of London where he worked on genetic modification of dendritic cells for tumour immunotherapy and obtained a PhD in 2010. Since 2011, he has been working with Sergio Quezada and Karl Peggs in the Cancer Immunotherapy group in the UCL Cancer Institute. The main interest of his group is to understand how the regulation of the immune response to cancer and what are the mechanisms that explain the response or resistance to tumour immunotherapy. Therefore, have been characterising the immune landscape in murine tumour models and in human cancers in order to design and understand the mechanisms of action of different forms of tumour immunotherapies. His particular interest focuses on engineering of antibodies targeting clinically relevant and novel immunomodulatory molecules.

INVITED SPEAKER

SII 2. Efficacy and Biomarker Analyses of the Use of Checkpoint Inhibition in Melanoma

Jeffrey Weber

Targeting of PD-1 in Melanoma: The immune checkpoint protein programmed death-1, or PD-1 has been shown to have an important role in controlling the adaptive immune response. An IgG4 human antibody against PD-1 was developed, and in a study of 107 metastatic melanoma patients administered nivolumab every 2 weeks for up to 96 weeks, median overall survival was 16.8 months across doses and 20.3 months at the 3 mg/kg dose, with 40% of patients alive at 3 years. Follow-up of those patients at ASCO 2015 showed a 32% response rate and a median survival of 20 months at 3 mg/kg, the dose in phase II and III studies. Nivolumab has also been tested in treatment-refractory melanoma patients, in which patients with ipilimumab-naïve or ipilimumab-refractory stage III or IV melanoma received nivolumab at 1, 3, or 10 mg/kg for up to 2 years. The response rate for both ipilimumab-refractory and –naïve patients was 25%, with responses lasting up to 140 weeks. Updated data on that trial at ASCO 2014 suggested that baseline elevated myeloid derived suppressor cells (MDSC) were associated with poor survival, and overall survival was similar for ipilimumab-naïve and refractory patients. The response rate for 92 ipilimumab refractory patients was 29%, with a median survival of 20.4 months. Baseline low MDSC, low tetramer positive antigen specific CD8 T cells, and low CD8+/CTLA-4+ T cells were associated with favorable survival. Increased stats3 signaling in T regulatory cells, and increased OX-40 expression on those cells was also associated with a favorable outcome. In that trial, occurrence of skin toxicity and vitiligo by week 12 was associated with superior PFS and OS. Toxicity in patients that had received ipilimumab did not differ from those who had never received IPI; even in those that had grades 3-4 irAEs after IPI< the side effects were not reproduced with subsequent nivolumab administration. For 22 all patients that reached the 2 and a half year term of that trial in CR, PR or stable disease, only 2 have relapsed to date and are both alive. An adjuvant trial in resected stage IV melanoma with nivolumab and a peptide vaccine suggested that the median PFS was quite long, with 10/33 patients relapsing at a median of 32 months of follow up, with only 6 deaths. Of 40 patients treated with adjuvant IPI plus NIVO, only one one patient has died and 4 relapsed with a median of 13 months of follow up. Recently phase III trial data published in Lancet Oncology suggest that in patients that progressed after ipilimumab, the response rate was 32% for nivolumab versus 11% for investigator choice chemotherapy, with fewer grade

3-4 drug related side effects (9% for nivolumab and 31% for chemotherapy) and long duration of response for the nivolumab-treated group. Responses were seen in BRAF mutated patients, those who had previously responded to ipilimumab, and in those whose tumors were PD-L1 negative. PD-L1 positive tumors were more likely to respond to treatment. These data led to nivolumab's approval for second line therapy of stage IV melanoma in the U.S. in December 2014. Sequential therapy with NIVO>IPI or IPI>NIVO showed similar rates of treatment-related grade 3/4 AEs occurred during both induction periods to week 25 for NIVO>IPI (50%) and IPI>NIVO (43%). In the updated analysis (minimum follow-up of 14 months), grade 3/4 AEs were 63% for NIVO>IPI and 50% in IPI>NIVO across all study periods, which led to discontinuation in 25% and 27% of pts, respectively. ORR was higher for NIVO>IPI patients than for IPI>NIVO patients (54% vs. 31%), with more complete responses (11% vs. 6%). A significant difference in OS was observed between NIVO>IPI andIPI>NIVO (hazard ratio: 0.48; 95% CI: 0.29, 0.80; P=0.0041). These data support the first line use of PD-1 blockade, with CTLA-4 blockade as a preferred second line treatment.

Jeffrey Weber earned his Ph.D. in molecular cell biology from Rockefeller University, and received his M.D. from New York University. He completed his internship and residency in Medicine at the University of California, San Diego, and his fellowship in Medical Oncology at the National Cancer Institute in Bethesda, MD. Currently, J.W. is senior faculty of NYU Langone Medical Center and its Laura and Isaac Perlmutter Cancer Center as Deputy Director of the Perlmutter Cancer Center and Co-Director of its melanoma program, and oversees its work in experimental therapeutics. Most recently, J.W. was Director of the Donald A. Adam Comprehensive Melanoma Research Center at Moffitt Cancer Center & Research Institute. His past experience includes clinical, research and teaching positions at the University of California, Irvine, and the University of Southern California where he was Chief of Medical Oncology and Associate Director for Clinical Research at the USC/Norris Comprehensive Cancer Center. A specialist in cancer immunotherapy. J.W. is principal investigator (PI) on several ongoing studies funded by the National Cancer Institute (NCI), including trials in clinical drug development, vaccines, and studies on autoimmunity and melanoma. He has been continuously NCI R01 funded for the last 16 years, and was the Principal Investigator and Director of the Moffitt Skin Cancer SPORE (P50) NCI grant. J.W. has published more than 100 articles in the top peer-reviewed journals in his field. J.W. research interests are in the field of immunotherapy for cancer. He has been a pioneer in the clinical advancement of antibodies that induce autoimmunity as a surrogate for clinical benefit in cancer, and the management of the autoimmune side effects.

SII 3. Functional capacity of tumor lysate-pulsed monocyte-derived dendritic cells inducing in vitro immune responses

Pinho A.C.[1,4], Lopes S.M.[1], Amado F.[1], Verdelho A.[2], Abreu de Sousa J.[3], Roncon S.[1]

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Introduction: Dendritic cells (DC) have been shown to be a promising adjuvant to initiate antitumor immune responses. Over the last years, several methods have been developed to isolate DC from cancer patients, ex vivo expand and pulse them, aiming to generate highly immunogenic clinical grade infusion products. In order to validate our previously established in vitro methodology for DC generation, the present study aims to assess the functional capacity of the DC final product inducing immune responses. Materials and Methods as part of an authorized pre-clinical study with solid tumors, four experiments were performed using tumor lysates (2 glioblastomas, 1 sarcoma and 1 breast cancer) for DC stimulation. DC were differentiated from monocytes obtained from peripheral blood (PB) and subsequently matured and pulsed with previously prepared tumor lysate, during 8 days of culture in cytokines-supplemented medium. Loaded mature DC (mDC) were evaluated for cell counting, viability, morphology and immunophenotype. DC functionality was evaluated via one-way MLR using peripheral blood mononuclear cells (PBMC) which were isolated from PB by density gradient centrifugation and pre-labeled with the 'green' fluorescent dye CFSE. The CFSE+ PBMC (responder cells) were co-cultured with mDC (stimulating cells) in a 96-well plate. PBMC without DC stimulation was used as negative control. In both MLR and negative control, the same tumor lysate used in DC stimulation was added or not. After 7 days of incubation at 37°C, 5% CO2, proliferation of responder cells was measured by flow cytometry. Results and Discussion The single-cell suspension obtained at the end of culture showed: numerous cells presenting extended and multiple dendrites; up-regulation of the characteristic maturation markers CD83/CD86; and down-regulation of CD14, marker of the precursor cells. Taken together these results prove the achievement of the DC maturation state. These final loaded mDC were assessed for the antigen

presentation skills by their in vitro allostimulatory capacity of PBMC. The average percentage of proliferating PBMC obtained when co-cultured with mDC was greater than that verified without DC stimulation (47.7±3.0% vs. 19.3±8.4% and 47.0±5.0% vs. 8.8±3.5%, with and without tumor lysate, respectively). Despite the small sample size, DC stimulation consistently induced more than double the PBMC proliferation. This study demonstrated the functionality of the mDC final product to induce in vitro immune responses. With our mDC manufacturing protocol validated, we are motivated to obtain the legal authorization for the implementation of this Advanced-Therapy Medicinal Product in clinical grade. It may be beneficial for the patients to have cell collection, production and administration available in the same hospital setting.

No conflict of interest

SII 4. PD-1 downregulation on Tregs during Tyrosine Kinase Inhibitor therapy in Chronic Myeloid Leukemia

Rodrigues-Santos P.[1,2,3], Almeida J.S.[2] , Couceiro P.[2,3] , Alves V.[1,3] , Růžičková L.[4] , Freitas-Tavares P.[4] , Santos-Rosa M.[1,3]

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Introduction: Programmed death-1 (PD-1) receptor and its ligands (PD-L1 and PD-L2) are involved in attenuating tumor immunity and facilitating tumor progression. PD-1, PD-L1 and PD-L2 therapeutic blocking agents have been reported to have significant antitumor effects. In chronic myeloid leukemia (CML), the expression of this receptor and its ligands is not fully characterized for the different subsets of cells of the immune system in which their expression is found constitutively or post-induction. In this study, we analyzed the expression of PD-1 and its ligands on regulatory T cells (Tregs) in chronic phase CML patients to understand the mechanisms underlying suppressor effects that inhibit the anti-leukemia immune response. Materials and Methods: Peripheral blood samples from chronic phase CML patients (n=50) under Interferon-alpha 2b (IFN-α 2b), imatinib, dasatinb, nilotinib, bosutinib and ponatinib therapy were analyzed by multi-parametric flow cytometry for the characterization of regulatory T cells and surface expression of PD-1. Buffy coats (n=13) from healthy blood donors were used as control. Cytokines and chemokines were evaluated in a 34-plex panel by xMAP technology (Luminex®). Gene expression analysis and miRNA profiling were also performed for these samples. Results: PD-1 Tregs were found significantly decreased (p<0.0001) in CML patients and down-regulation of this receptor was also observed (p<0.01). Naïve and memory Treg subsets were equally affected. No significant alterations were observed for PD-L1 and PD-L2 ligands. Although TGF-β and IL-10 production were not significantly altered by down-regulation of PD-1 in Tregs, the overall effect of tyrosine kinase inhibitor (TKI) therapy suggests a negative impact in these cells concerning the anti-leukemic immune response. Discussion: Regulatory T cells represent a major population of suppressors in the immune response against leukemia. Down-regulation of PD-1 receptor in Tregs during chronic phase CML reinforces the notion that discontinuation of treatment must be carefully evaluated beforehand, since these suppressor cells could permit the proliferation of existent residual leukemic cells. Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

No conflict of interest

ABSTRACTS

INVITED SPEAKER

SIII 1. Molecular tumour response to accelerated ions and role of cellular modifying factors.

Rolf Lewensohn

(no abstract available)

Rolf Lewensohn has a scientific background in DNA repair apoptotic signalling in cancer as related to cancer therapy. His research focuses on the sensitivity of human tumours to radiation, as well as conventional and experimental chemotherapeutic drugs, with regard to the role of growth factor, DNA repair, and apoptotic signalling. He and his group aim to develop new oncological treatments in the form of novel drug candidates and precision radiotherapy, with a personalized cancer medicine approach. The general interest of the research group is the development of novel treatments (both drugs and radiotherapy), mainly focused on lung cancer and furthermore breast, renal, and cervical cancer, multiple myeloma, and acute myeloid leukemia. From all types of malignancies, lung cancer is the leading cause of cancer-related mortality. In parallel, the group runs clinical trials on lung cancer at the Karolinska university hospital, in collaboration with pharmaceutical partners. Furthermore, the group has a leading role in the EurocanPlatform, an EU-funded project to bring together 28 European cancer institutions and organizations, with the aim to improve outcomes for cancer patients and reduce mortality. Within the Karolinska Institute, and Karolinska university hospital, R.L. is the primary initiator of the development of a personalized cancer medicine program. The ultimate goal of this program is to individualize therapy at all stages of disease by quickly and efficiently translating the latest scientific advances into concrete improvements of the care for cancer patients.

INVITED SPEAKER

SIII 2. Focus on the combination of DNA repair inhibitors with radiotherapy

Marie Dutreix

 A majority of cancers are today treated by agents causing DNA damage as the chemotherapies (CT) and the radiotherapy (RT). However the therapeutic index of these treatments is limited because of the intrinsic or acquired resistance of tumors and toxicity of treatments on healthy tissues which limits the using dose. Resistance to RT is mainly related to the enhanced activity of DNA repair allowing cancer cells to survive to the damage induced by the treatment. It is thus essential to develop new therapeutic agents inhibiting the pathways of DNA repair to restore the sensibility. However, activities, as DNA repair, are essential for species to survive and maintain their genome integrity, and thereby display functional plasticity and redundancy. This is the cause of failure of many targeted inhibitors as alternative pathways rely the function inactivated by the hit of the targeted enzyme. To overcome this limitation we have developed a new concept of multi-pathway inhibitors, the siDNAs. The siDNA are designed to globally inhibit the DSB repair machinery, by preventing recruitment of enzymes involved in DSB and SSB break repair at damage site. They are small molecules mimicking DNA damage, binding and activating PARP and DNA-PK signaling enzymes. The resulting "false" signal prevents recruitment of repair enzymes at damage site and therefore inhibits repair and increase sensitivity to irradiation. The radiosensitisation by the Dbait,, first siDNA developed, was observed in many tumor types but not in healthy tissues. This selectivity was observed in cell cultures, in xenografted animals and in patients (DRIIM trial). We will discuss preliminary results to explain the differential effect of siDNA in tumor and normal cells.

SIII 3. TERT promoter mutations in thyroid carcinoma from childhood irradiated tinea capitis patients

Paula Boaventura [1,2], Rui Batista [1,2], Marta Reis [1,2], Adélia Mendes [1,2], Catarina Eloy [1,2], Manuel Sobrinho-Simões [1,2,3,4], Paula Soares [1,2 3]

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Introduction: - TERT promoter mutations either with or without BRAF mutations have been studied in sporadic thyroid cancers, and in thyroid cancers after Chernobyl burnout, but not in the X-ray irradiation context. Since a molecular signature for radiation-induced thyroid cancer is not yet available we decided to evaluate the recently discovered TERT promoter mutations in the low dose radiation tinea capitis model.Material and methods – Thirty four samples of thyroid carcinoma (from 27 patients), one case of a well differentiated tumour with unknown malignant potential (WDTUMP) and 28 cases of follicular adenoma (from 27 patients), diagnosed in a Portuguese tinea capitis cohort of 1375 patients, were studied. Blood samples were obtained for all the patients. The lesions and the blood were screened for the TERT promoter mutations by PCR amplification followed by Sanger sequencing. Results and Discussion –TERT promoter mutations were detected in four of the 34 thyroid carcinomas (11.8%) and in five of the 28 adenomas (17.9%). The WDTUMP case also presented the mutation. All the mutations were the -124 T<C except for three tumours (two carcinomas and one adenoma) which presented the tandem mutation -124/-125 (3/10, 30.0%). No mutations were found in the blood.

In our series, TERT promoter mutations were a fairly common event in benign and malignant thyroid tumours in an X-ray irradiated cohort, differing from the sporadic context where it is a very rare event in benign tumours. It also differs from the Chernobyl setting in which such mutations were absent. Moreover, we found the tandem mutation -124/-125 that had not been previously described in thyroid tumours. Our data suggest that the pattern of TERT promoter mutations in the different settings possibly reflect a different etiopathogenic background.

No conflict of interest

SIII 4. Macrophages modulate colorectal cancer cell response to radiation

Pinto A. T.[1,2,3], Pinto M.L.[1,2,4], Velho S.[1,5], Pinto M. T. [1,5], Figueira R. [6], Monteiro A.[6], Marques M.[6], Seruca R.[1,5,7], Oliveira M. J.[1,2,7], Rocha S. [8]

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Introduction: When a tumour is subjected to radiotherapy, not only cancer but also tumour-associated cells are exposed to ionizing radiation. In many tumours, macrophages constitute the most representative population of tumour-associated cells, playing a role in tumour progression and response to therapy. The present work aimed to evaluate the effect of ionizing radiation on macrophage-cancer cell crosstalk, and to elucidate how macrophages may modulate cancer cell response to radiation, in cells with distinct radiation sensitivities. Materials and Methods To address these questions, we established monocultures and indirect co-cultures of human monocyte-derived macrophages with RKO or SW1463 colorectal cancer cell lines, which exhibit lower or higher radiation sensitivity, respectively. Mono- and co-cultures were then irradiated with 5 cumulative doses, in a similar fractionated scheme to that used during cancer patients´ treatment (2 Gy/fraction/day). Results and Discussion Our results demonstrated that macrophages sensitize RKO to radiation-induced apoptosis, while protecting SW1463 cells. In order to explain this observation, we focused on cancer cells mRNA expression levels of metabolism- and survival-related genes, which we found to be generally higher in SW1463 than in RKO, upon co-culture with macrophages. Conversely, the influence of cancer cells on the expression of pro- and anti-inflammatory macrophage markers, upon ionizing radiation exposure, was also evaluated. Our data demonstrated that the response of both macrophages and cancer cells to radiation could be mutually influenced. Finally, we demonstrated that conditioned medium from irradiated co-cultures promoted non-irradiated RKO cell migration and invasion. Our data suggest that macrophages increase RKO radiosensitivity, while promoting SW1463 radioresistance, which is of major interest when considering the development of therapies adjuvant to radiotherapy. The establishment of macrophage-cancer cell co-cultures contributed to elucidate the role of this immune cell population on cancer cell response to ionizing radiation.

No conflict of interest.

ABSTRACTS

INVITED SPEAKER

SIV 1. *The continued role of AR in castration-resistant prostate cancer.*

Wytske M. van Weerden

(no abstract available)

Dr. Wytske M. van Weerden is a senior scientist in biology and staff member of the department of Urology. She received her PhD in 1992 at the Erasmus University Rotterdam after which she continued her research on prostate cancer. She has centered her preclinical research group around the unique panel of patient-derived xenografts (PDXs) models for prostate cancer, which she extended to also include castration and chemotherapy-resistant models. Her group is dedicated to the following research interests: prostate cancer modeling, with special attention to the development of novel clinically relevant PDX models of resistance to contemporary therapies, modeling of (bone)metastasis, ex vivo tissue slices and 3D co-cultivation models; resistance mechanisms of hormone - and chemoresistant CRPC, with special focus on androgen receptor and PI3K-targeted therapies, steroidogenic bypass mechanisms as well as drug transporters; and finally, multimodality prostate-targeted molecular imaging using prostate specific nanobodies. Furthermore, her group is actively involved in evaluating novel (targeted) therapies and testing of novel compounds, the latter in close collaboration with the pharmaceutic industry. The research group collaborates nationally (CTMM-PCMM, the Dutch Cancer Society (KWF)-Alpe D'HuZes and several Dutch pharmaceutic companies) and internationally in projects funded by the EU (Framework -7 GIANT, IMI-PREDECT), global foundations like Movember, and several international pharmaceutic companies.

INVITED SPEAKER

SIV 2. *The role of the AR in advanced breast cancer*

Tiffany A. Traina

(no abstract available)

Dr. Traina is a member of the Breast Medicine Service at Memorial Sloan Kettering Cancer Center, New York City, and Assistant Professor for the Department of Medicine at Weill Cornell Medical College, New York City. She received her doctor of medicine from Weill Cornell Medical College, completing her Internal Medicine training at The New York Presbyterian Hospital-Cornell University campus and her Medical Oncology and Hematology training at Memorial Sloan Kettering Cancer Center. Her primary research interests focus on the development of improved therapies for patients with breast cancer with a particular focus on triple negative breast cancer, the androgen receptor and the use of mathematical modeling to optimize drug delivery schedules. She serves on the ASCO Scientific Program Committee for the Triple Negative Breast Cancer/Cytotoxics/Local Therapy track and is a member of the Translational Breast Cancer Research Consortium Triple Negative Breast Cancer Working Group. She has been recognized for her academic, patient care and community service efforts including the Hally Yaccino Steiner Award from the Susan G. Komen Breast Cancer Foundation, Teacher Appreciation awards from MSKCC and Weill Cornell Medical College, the 2014 Hero Award from the Triple Negative Breast Cancer Foundation and the Outstanding Service Award from the Junior League.

SIV 3. Estrogen-depeendent breast cancer: The involvement of androgen receptor in exemestane-acquired resistance

Amaral C.[1], Correia-da-Siva G.[1] , Roleira F.M.F.[2,3] , Tavares-da-Silva E[-2,3] , Teixeira N.[1]

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Introduction: The third-generation of aromatase inhibitors (AIs) is one of the therapeutic approaches used for estrogen-receptor positive (ER+) breast cancer, being Exemestane (Exe) the steroidal AI used in clinic [1]. One of the major drawbacks in endocrine therapy is the occurrence of AIs-acquired resistance. Therefore, it is needed to find new targets to improve breast cancer treatment. It is known that AIs-acquired resistance may be associated to high androgen levels or high androgen receptor (AR) expression [2]. In that way, this work will investigate the involvement and the biological significance of AR and androgens in Exe-treated sensitive and resistant ER+ breast cancer cells. Material and Methods: In order to understand the involvement of AR, it was investigated in an AIs-resistant breast cancer cell line (LTEDaro) and in a sensitive ER+ breast cancer cell line that overexpress aromatase (MCF-7aro), the in vitro effects of an AR antagonist (Casodex, CDX) in Exe-treated cells. The cell viability effects were studied using MTT assay. The induced cell death was explored by evaluating caspases-9 and -7 activities. Additionally, to understand if Exe increases androgen levels, it was evaluated the activity of 5α-reductase (5α-R), the enzyme responsible of androgen synthesis and quantified the levels of the main androgen, dihydrotestosterone (DHT), using a sensitive gas chromatography-mass spectrometry (GC-MS) method [3]. Results and Discussion: The results indicate that Exe increases the 5α-R activity and consequently the DHT levels, which suggests that Exe may have an androgen-dependent effect. Moreover, by blocking AR with CDX, it was observed an increase in the reduction of viability of Exe-treated MCF-7aro cells as well as caspases-9 and -7 activities, when comparing to Exe. Furthermore, it was found that CDX can decrease viability of Exe-treated LTEDaro cells, being the behavior similar to the sensitive cells, by increasing caspases-9 and -7 activities. All together this study indicates that AR may be involved in Exe-acquired resistance and that by targeting AR with antagonists it is possible to sensitize resistant cells to Exe-treatment, promoting cell death by apoptosis. Thus, this work contributes to understanding the link between androgens/AR and AIs-resistance and will highlight new targets to improve breast cancer treatment. Acknowledgements: FCT: Amaral C. grant (SFRH/BPD/98304/2013) and (UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728); Prof. Shiuan Chen (Beckman Research Institute, USA) for MCF-7aro/LTEDaro cells. [1] Amaral C., et al. (2012) PLoS ONE; 7(8): e42398. [2] Ma C.X., et al., (2015) Nat Rev Cancer; 15(5): 261-75. [3] Amaral C., et al. (2013) Talanta; 107: 154-61

No conflict of interest

SIV 4. Androgen Receptor mediates castration-resistant prostate cancer cells response to the DNMT inhibitor Hydralazine

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Introduction: Aberrant DNA methylation plays a critical role in prostate cancer (PCa) development and progression, particularly in the acquisition of the castration resistance phenotype (CRPC). Therefore, DNA methylation inhibitors (DNMTi) might constitute promising alternative therapies for PCa management. In a previous study we found that hydralazine, a non-nucleoside DNMTi, was able to attenuate the malignant

phenotype of PCa cells. Interestingly, the phenotypic effects of hydralazine were particularly impressive in DU145 cell line, which is a CRPC cell line due to Androgen Receptor (AR) promoter methylation. Herein we investigated the mechanism of action of hydralazine in CRPC cell lines and its relation to AR in order to determine whether the subset of PCa patients that acquire the CRPC phenotype due to loss of AR expression may benefit from treatment with this drug. Material and Methods: AR was stably re-expressed in DU145 and PC-3 cell lines. After confirming AR re-expression by qRT-PCR and Western blot (WB), these cell lines were exposed to hydralazine for three days and several phenotypic assays were performed. A protein array was performed and the data confirmed by WB. EGFR expression was assessed by qRT-PCR. DU145 cell line was exposed to hydralazine followed by bicalutamide and phenotypic assays were performed. Results: After exposure of these cells to hydralazine both DU145_AR or PC-3_AR were less sensitive to this drug than the respective control cell lines. Moreover, hydralazine exposure associated with increased AR expression. A protein array analysis suggested that hydralazine induced a blockage of EGF receptor signaling pathway, which appears to be mediated by AR re-expression. In fact, exposure of both AR induced and control DU145 cells to hydralazine led to significant decrease in EGFR expression. Importantly, the combined exposure of DU145 cells to hydralazine and the AR-antagonist bicalutamide potentiated the reduction of tumor cell growth. Discussion: Our data indicates that hydralazine response might be mediated by a loop of AR and EGFR activities.

No conflict of interest

ABSTRACTS

INVITED SPEAKER

SV 1. *Molecular features of SPOP mutant/ CHD1 deleted prostate cancer.*

Gunther Boysen

Recent DNA and RNA sequencing studies of localized and advanced prostate cancer identified distinct novel molecular subclasses of this disease. Mutations in SPOP gene occur frequently and often in combination with deletions of the chromatin remodeler CHD1. Functional analysis of this prostate cancer subclass revealed striking molecular features including increased genomic instability and aberrant androgen transcriptional activity. However, the molecular mechanisms underlying these abnormalities are only partially understood and translation of these findings into the clinic is lacking. I will summarize the results of our preclinical studies, which focus on understanding the functional consequences of CHD1 and SPOP loss of function in prostate carcinogenesis, particularly in late stage disease, and will discuss their potential use as biomarkers for rationally designed clinical trials of targeted therapies in prostate cancer.

Gunther holds the title of Doctor of Science (Dr. Sc. ETH Zurich) from the Swiss Federal Institute of Technology (ETH) Zurich in Molecular and Cellular Biology. Currently he is a Marie Curie postdoctoral fellow at the Institute of Cancer Research in London, UK. He joined the team of Professor Johann de Bono in April 2014 after moving from Professor Mark Rubin's group at Weill Cornell Medical College in New York. His research focuses on understanding the impact of genetic alterations on tumor progression and their role as potential drug targets. G.B. recent work unraveled the mechanism leading to genomic instability in a subset of prostate cancer – potentially targetable with DNA damaging drugs. In addition, G.B. is leading efforts to develop better models for castration resistant prostate cancer, which include the organoid technology, genomic-engineering by CRISPR/CAS9 and patient derived xenograft mouse models. His research has been awarded with the Charles Rodolphe Brupbacher Young Investigator Award (2007), Department of Medicine Grant for Innovative Research (2013), Marie Curie International Incoming Fellowship (2014) and the ICR Dean's award (2015).

INVITED SPEAKER

SV 2. *Tackling intratumour heterogeneity in brain tumors*

Joan Seoane

(no abstract available)

Joan Seoane obtained his PhD in Biochemistry and Molecular Biology from the University of Barcelona in 1998. He has a BSc degree in Chemistry, branch of Biochemistry and Molecular Biology. J.S. joined the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York as a post-doctoral fellow in 1998. From 1998 to 2001, he worked as a Research Fellow at this institution and subsequently, from 2001 to 2003, as a Research Associate. He was appointed ICREA Research Professor in 2004 and established his own Group back in Barcelona at the Vall d'Hebron Institute of Oncology (VHIO). In 2007, he became a member of the Young Investigator EMBO program and the recipient of a European Research Council grant. In 2008, he became Board member of the European Association of Cancer Research (EACR). Since 2011, J.S. is the Director of the Translational Research program at VHIO within the Vall d'Hebron University Hospital. He is the recipient of several awards such as the MSKCC research fellow award, the Banc Sabadell biomedical award, the Josef Steiner award and the Drs. Diz-Pintado award. Research Interests: J.S. main objective is to understand the molecular mechanisms involved in the initiation and progression of cancer, including the concept of intratumor heterogeneity. Specifically, his research is focused on the study of brain tumors. The understanding of the molecular mechanisms that govern brain tumors is required in order to design rational, specific and successful therapeutic approaches.

SV 3. Autofluorescence as a new biomarker to identify Glioblastoma Stem Cells

Vieira de Castro, J.[1,2] , Miranda-Lorenzo I. [3] , Cerqueira M.T. [2,4] , Pinto A.A [5] , Heeschen C. [3] , Costa B.M. [1,2]

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Introduction: Glioblastoma stem cells (GSCs) have been associated with tumor initiation, progression and recurrence, as well as with therapy resistance. Therefore, to achieve improved responses of glioblastoma patients, the GSC subpopulation must be specifically targeted. Although several cell surface markers (e.g. CD133, CD15) have been used to isolate GSCs, their expression is variable and not exclusive. Recently, it was demonstrated, in different human epithelial tumor types, that a subpopulation of cells with CSCs features presented an autofluorescent subcellular compartment. Thus, we aimed to validate autofluorescence as a new biomarker to improve GSCs identification/isolation. Materials and methods: The autofluorescent population was detected in both glioblastoma tumors and primary glioblastoma cell lines by flow cytometry with a 488 nm blue laser and selected as the intersection between filters 530/40 and 580/30. Neurospheres were generated in Neurobasal medium supplemented with 1x B27, 20 ng/mL of b-FGF and EGF for 14 days. For serial passag¬ing, 14-d-old neurospheres were dissociated into single cells and then re-cultured for 14 additional days. The expression of pluripotency-associated genes and stem cell surface markers were performed by qPCR and flow cytometry, respectively. In order to evaluate the percentage of autofluorescent cells after temozolomide (TMZ) treatment or exposure to radiation, primary glioblastoma cells were treated with the IC50 of TMZ for 3, 6 and 9 days, or were irradiated with 2, 4, 6, 8 and 10 Gy. Results and discussion: The subpopulation of autofluorescent cells presented typical GSCs features, including increased radio- and chemotherapy resistance, enriched capacity to grow as neurospheres, and higher expression of stem cell markers and pluripotency-associated genes. Autofluorescent cells also formed significantly more neurospheres over three generations, whereas non-autofluorescent cells appeared to lose this capacity. Critically, the underlying mechanism of the autofluorescent phenotype was the accumulation of riboflavin in cytoplasmic vesicles bearing ATP-dependent ABCG2 transporters, which occurred exclusively in GSCs. In this work, we have identified an intrinsic autofluorescent phenotype in GSCs that can be used as a biomarker. Our findings will allow us to overcome problems associated with the use of cell surface markers to more easily and specifically isolate GSCs, as well as to search for new biomarkers by characterizing the cell surface proteome of this autofluorescent GSCs.

No conflict of interest

SV 4. P-cadherin, a therapy predictive biomarker for Dasatinib in basal-like triple negative breast cancer

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Introduction: Basal-like triple-negative breast cancer (BL-TNBC) is a poor prognosis subgroup of carcinomas that still lack target therapies and accurate biomarkers for treatment selection. Our group has been involved in identifying new molecular targets for BL-TNBC, such as P-cadherin (Pcad). This protein is overexpressed in TNBC and its expression is significantly associated to poor patient survival. Pcad overexpression promotes in vitro cell migration and invasion, through activation of Src Family kinase (SFK) signaling pathway. Therefore, we

decided to test the treatment of BL-TNBC with the FDA approved SFK inhibitor, Dasatinib, and understand whether Pcad could be used as stratification therapy biomarker. MATHERIALS AND METHODS: We characterized Pcad and SFK expression in a series of invasive BC. In vitro invasive capacity was analysed by embedding BC cellular aggregoids (manipulated for CDH3 expression and/or treated with Dasatinib) in 3D collagen type I matrix. In vivo xenograft mouse models were used to evaluate the impact of Dasatinib on tumor growth and survival. RESULTS/DISCUSSION: To clarify the association between Pcad and SFK activation, we analysed CDH3 expression and SFK associated genes (SRC, YES1, FYN, LYN, LCK, CSK) in a public database of BC cell lines. A significant positive association between CDH3 expression and SFK gene signature was found and was further confirmed using in vitro assays and primary tumors samples. After, we evaluated the potential of Dasatinib in the inhibition of downstream signalling induced by Pcad overexpression. Our results showed that Dasatinib inhibits Pcad/SFK signalling significantly preventing in vitro invasive activity, as well as in vivo tumorigenic and metastatic ability of Pcad overexpressing BL-TNBC cells. Interestingly, Dasatinib treated mice showed an improvement of mice wellbeing and increased overall survival. Accordingly, by analyzing a panel of BC and prostate cancer cell lines, a significant positive association was observed between CDH3 expression and Dasatinib response, showing that CDH3 expression is able to predict sensitivity/resistance of cancer cells to Dasatinib. Overall, our findings pinpoint Pcad as a pharmacologically predictive biomarker to guide the selection of patients for Dasatinib-targeted therapy, opening a new therapeutic opportunity to BL-TNBC treatment.

No conflict of interest

ABSTRACTS

ASPIC SPONSORED LECTURE

PL 1. *Mapping the development of Portuguese clinical research in oncology.*

Alberto Cambrosio

(no abstract available)

A.C. is a professor at McGill University since 1990. A.C. area of expertise lies at the crossroads of medical sociology and the sociology of science and technology. His work focuses on the "material culture" of biomedical practices, and in particular on the study of the application of modern biological techniques to the diagnosis and the therapy of cancer, the comparative (North-America - Europe) development of cancer clinical trials, and the role of visual imagery in the development of immunology. He is especially concerned, in how biomedicine has come to grips with the multiple and ubiquitous cultural, social and practical differences and variations with which it is increasingly confronted. More in particular, he is interested in the creation of institutions and instruments to manage these differences and generate consensus, however partial or temporary in nature, and thus with the social and historical dynamics of biomedical regulation, objectification and standardization. A.C. most recent project (supported by grants from the Social Sciences and Humanities Research Council of Canada, the Fonds Québécois de Recherchesur la Société et la Culture and the Canadian Institutes of Health Research) examines 'genomics in action', i.e., as applied to concrete instances of medical work, by investigating public, academic and commercial programs that capitalize on the therapeutic insights offered by the new molecular genetics of cancer.

ASEICA SPONSORED LECTURE

PL 2. *Lung cancer precision treatment.*

Luis Paz Ares

(no abstract available)

Dr Paz-Ares is currently Associate Professor of Medicine (Complutense University), Chair of the Oncology Department at the Hospital Universitario Doce de Octubre, and Head of the Lung Cancer Unit at the CNIO (Centro Nacional de Investigaciones Oncologicas), Madrid Spain. He obtained his medical degree in 1986 from the Universidad Autonoma, where he also completed his studies for a PhD in Oncology in 1993. In 1995 he obtained his MSc in Clinical Pharmacology from the University of Glasgow, UK, and in 2003 he was awarded a Master degree in Clinical Units Management from the Universidad UNED, Madrid. L.P.A. originally trained in Medical Oncology and in 1993 he took up a post as a European Society for Medical Oncology (ESMO) Fellow in New Drug Development at the CRC Department of Medical Oncology in Glasgow. In 1995 he moved to the Doce de Octubre University Hospital, Madrid and was Head of the Lung and GU Tumours and Drug Development Units. He moved to Seville in 2007 to chair the Medical Oncology Department at the Virgen del Rocío University Hospital until December 2014 when he took his current position. His main research interests include the testing and development of novel therapies, and lung cancer. He is the author of more than 180 papers in peer-reviewed journals, as well as many book chapters. He is an active member of various scientific societies (including ASCO, ESMO, IASLC and other) and collaborative groups (European Organisation for Research and Treatment of Cancer [EORTC], the Spanish Lung Cancer Group and the International Germ Cell Cancer Collaborative Group).

PL 3. *RAS and RAF signalling in melanoma: from basic biology to clinical responses.*

<u>Richard Marais</u>

(no abstract available)

Richard Marais obtained his BSc in Genetics and Microbiology from the University College London in 1985, after which he undertook his PhD in Comparative Studies on Protein Kinase C Isotypes at the Ludwig Institute for Cancer Research in London, which he completed in 1989. He then worked as a Postdoctoral Research Fellow at the Imperial Cancer Research Fund, London, until 1993. R.M. then moved to The Institute of Cancer Research (ICR) in London as an Independent Postdoctoral Research Fellow. It was at the ICR where R.M. spent the next 19 years of his career focusing on cell signalling in melanoma, developing a particular interest in the role of oncogenic BRAF. During his time at the ICR, R.M. progressed to Team Leader of the Signal Transduction Team in 1998, then to Professor of Molecular Oncology in 2007, to Deputy Chair, Section of Cell and Molecular Biology, in 2008, and finally to the Division Head, Division of Cancer Biology, in 2011. In 2007, he was elected Fellow of the Academy of Medical Sciences. In 2009, he was elected Fellow of the European Academy of Cancer Sciences and became an EMBO member. In 2011, he received the Society for Melanoma Research Estella Medrano Memorial Award for outstanding contributions to melanoma research. R.M. became Director of the CRUK Manchester Institute in February 2012 where he also continues to head his Molecular Oncology Group. In 2015, R.M. was elected to the Academia Europaea.

A1. Identification and characterization of novel tumor suppressor genes implicated in lung carcinogenesis

Pereira C.[1], Gimenez-Xavier P.[1], Gomez G.[2], Condom E.[3], Villanueva A.[4], Pisano D.[2], Sanchez-Cespedes M.[1]

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Introduction: The poor prognosis and lack of effective therapies throw lung cancer (LC) to the deadliest cancer worldwide. The mutational spectrum is one of the keys to determine tumor development, and therefore, the discovery of LC-related genes (mainly oncogenes and tumor suppressors) has been crucial to the development of targeted drugs, which are now available for patients bearing specific mutational patterns. MATERIAL AND METHODS: Here, aiming to characterize novel tumor suppressor genes (TSG), 8 tumorgrafts were generated to perform exome and transcriptome sequencing. Data was scrutinized, by focusing on the subset of genes holding mutations that lead to biallelic gene inactivation. B2M gene (coding for the beta2-microglobulin) was selected and its mutation rate was determined in LC. Subsequently, its biological function was addressed through assays carried out after its restoration in gene-deficient cell line models. RESULTS AND DISCUSSION: From the 4428 mutated genes across all tumorgrafts, we selected B2M, which was found to be altered in 5.1% in 79 tested LC cell lines and in 4.0% in a panel of 174 lung primary tumors. In vitro experiments demonstrated that B2M is critical to stabilize and transport the MHC class I complex to the cell membrane. Immunohistochemistry analysis of tissue microarrays showed that the complex is absent or downregulated in almost 50% of lung tumors. MHC-I is crucial to the recognition and destruction of cancer cells by cytotoxic T cells and indeed, the presence of the complex correlated with higher levels of CD8 T cell infiltration in lung tumor nests. Additionally, gene expression data showed that the restoration of B2M enhanced the expression of genes involved in immune response, indicating that somehow, cells become more prone to respond to immune stimuli. Together, these data suggest that B2M has tumor suppressive functions in lung cancer, by enhancing the ability of tumors to escape the action of the immune system.

No conflict of interest

A2. Regulation of WNT6 by HOXA9 in glioblastoma: functional and clinical relevance

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Background: Glioblastoma (GBM) is the most common and most malignant type of glioma, a heterogeneous group of primary brain tumors. While the clinical outcome of GBM patients is unpredictable, patients are equally treated with a standardized approach. Thus, the identification of new biomarkers is crucial. HOXA9

overexpression in GBM is associated with poor prognosis and a more aggressive tumor phenotype. We recently found that HOXA9 transcriptionally activates the WNT pathway; here, we explore how WNT6, a WNT ligand/activator, may contribute to the malignant behavior of GBM. Material and Methods: Gene set enrichment analysis (GSEA) was used to query the HOXA9 transcriptome. Quantitative PCR, Western blot, chromatin immunoprecipitation (ChIP), methylation-specific PCR (MSP), and immunohistochemistry were performed in GBM cell lines, in vivo xenografts, or in patient samples to study WNT6 at various molecular levels. The functional effects of WNT6 in cell viability (MTT/Trypan blue), invasion (transwell matrigel), cell death after treatment with temozolomide (TMZ; Annexin/PI staining) and stemness capacity (limiting dilution assay) were assessed after silencing WNT6 with shRNA. U373+/-WNT6 cells were intra-cranially implanted in NSG mice to evaluate implications in survival. TCGA dataset was assessed for WNT6 status and clinicopathological correlations. Results and Discussion: We found that the Wnt pathway is over-activated in HOXA9-positive GBM cells. Specifically, WNT6 is a direct transcriptional target of HOXA9 and is overexpressed in a subset of GBM patients. Additionally, we observed that WNT6 expression correlates with higher glioma grades and with the GBM proneural subtype, whose patients do not benefit from more intensive therapies. Interestingly, we demonstrated that WNT6 expression is also regulated by DNA methylation in GBM patients. In vitro, WNT6-positive cells showed increased viability, migration, invasion and resistance to TMZ, and decreased cell death, when comparing to their negative counterparts. When cultured in stem-cell conditions, WNT6-positive cells show increased viability and capacity to form neurospheres than WNT6-negative cells. In addition, mice bearing WNT6-positive tumors presented faster glioma-related symptomatology and a significantly shorter overall survival (p=0.0042). Importantly, we provide the first evidence of the clinical prognostic value of WNT6 in GBM patients from TCGA and at the protein level in a cohort of Brazilians patients, implicating high levels of WNT6 as a novel independent negative prognostic marker. Together, our findings provide mechanistic, functional and prognostic insights into the role of WNT6 in GBM, creating opportunities to novel therapeutic approaches to treat this highly-aggressive cancer.

No conflict of interest

A3. Glycomic analysis discloses glycans as modulators of RON receptor tyrosine kinase activation in gastric cancer

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Introduction: In cancer the disruption of the glycosylation machinery leads to aberrant expression of short truncated carbohydrate chains, known as cancer-associated simple carbohydrate antigens, and to increased expression of terminal sialylated chains. These cancer-associated antigens are detected in different types of carcinomas and are associated with disease prognosis, constituting a pool of potential cancer biomarkers, especially when combined with information on the carrying proteins. Particularly terminal sialylation of glycans precludes further chain elongation and leads to the biosynthesis of cancer relevant epitopes such as sialyl-Lewis X (SLeX). In the present work, we have performed a detailed analysis of gastric carcinoma cells overexpressing the human α2,3-sialyltransferase ST3GAL4. Material and Methods: MKN45 gastric carcinoma cells stably transfected with the sialyltransferase ST3GAL4 were established as a model overexpressing sialylated terminal glycans and were compared to control clones. We have evaluated at the structural level the glycome and the sialoproteome of this gastric cancer cell lines applying liquid chromatography and mass spectrometry. We further validated an identified target by complementary techniques such as Western blotting, immunofluorescence, immunohistochemistry and proximity ligation assay in the cell line model and in

gastric tumors. Results and Discussion: Our results showed that ST3GAL4 overexpression leads to several glycosylation alterations, including reduced O-glycan extension and decreased bisected and increased branched N-glycans. A shift from α2-6 towards α2-3 linked sialylated N-glycans was also observed. Sialoproteomic analysis identified 47 proteins with significantly increased sialylated N-glycans. These included integrins, insulin receptor, carcinoembryonic antigens and RON receptor tyrosine kinase. Further analysis of RON confirmed its modification with SLeX and its concomitant activation. SLeX and RON co-expression was further validated in gastric tumors. This study shows that aberrant glycosylation of the RON receptor constitutes an important mechanism of oncogenic activation. Reference: Mereiter, S., et al. "Glycomic analysis of gastric carcinoma cells discloses glycans as modulators of RON receptor tyrosine kinase activation in cancer." Biochimica et Biophysica Acta (BBA)-General Subjects (2015).

No conflict of interest

A4. pmTOR is a marker of aggressiveness in papillary thyroid carcinomas

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mTOR pathway overactivation is found in a variety of human malignancies, being associated in some instances with distant metastases and poor prognosis. mTOR pathway activation has already been observed in thyroid cancer, but the biological consequences regarding tumor behavior and patient prognosis remain poorly explored. In order to evaluate mTOR pathway associations with clinicopathological and molecular features as well as with patients' prognosis, we analyzed, by immunohistochemistry, the expression of pmTOR and pS6 (as readouts of the pathway) in a series of 191 cases of papillary thyroid carcinoma. pmTOR expression was significantly associated with distant metastases and persistence of disease. Cases with higher pmTOR expression had significantly lower sodium iodide symporter expression and were submitted to more 131I treatments and higher cumulative doses of radioactive iodine. Furthermore, positive pmTOR expression showed to be an independent risk factor for distant metastases. On the other hand, pS6 was significantly associated with absence of extrathyroidal extension, well defined tumor margins and wild type BRAF status. There was no correlation between the expression of pmTOR and pS6. pmTOR expression is an indicator of clinically aggressive metastatic thyroid tumors, being possibly implicated in refractoriness to radioactive therapy, while pS6 expression is associated with less aggressive pathological features. Further studies are needed in order to understand better the biological consequences of the mTOR pathway activation in thyroid cancer behavior, namely the contribution of other pmTOR downstream effectors.

No conflict of interest

A5. A dual role of nrarp in T-Cell acute lymphoblastic leukemia pathogenesis

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe

toxicity. We have shown 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. Currently we are investigating the role of NRARP in human T-ALL cell growth and survival and its therapeutic potential in T-ALL. Materials and Methods: mRNA and protein expression were determined by real time-PCR and western blot analyses. In vitro functional evaluation of NRARP in T-ALL cell lines was performed by flow cytometry analysis of proliferation and viability upon NRARP overexpression using lentiviruses. Results and discussion: We started by characterizing NRARP expression in human T-ALL cell lines and compared it with the expression of NRARP in human thymocytes. We found that NRARP protein levels are significantly increased in T-ALL cells. This result, although consistent with the fact that NRARP is a transcriptional target of NOTCH, suggests that NRARP is not sufficient to block NOTCH oncogenic signals. To test this hypothesis, we overexpressed NRARP in human T-ALL cell lines. Curiously, NRARP overexpression blocks the expansion of the T-ALL cell lines that display NOTCH1-activating mutations (n=4) but promotes the expansion of the T-ALL cells without NOTCH mutations (n=3). It is known that NRARP positively regulates LEF1, a DNA binding transcription factor acting downstream of the WNT signaling pathway, shown to induce the malignant transformation of murine thymocytes. Very interestingly, immunoprecipitation analyses revealed that whereas in NOTCH1-mutant cell lines NRARP associates with a short isoform of LEF1, described as a WNT signaling antagonist; in NOTCH1-WT cell lines it binds to the full length LEF1. Taken together our preliminary results suggest that NRARP may play a dual role in T-ALL pathogenesis, regulating both NOTCH and WNT/LEF1 pathways, with opposite functional effects on leukemia cells depending on NOTCH mutational status and signaling levels. This dual role may have important biological and therapeutic implications.

No conflict of interest

A6. -Bromopyruvate induces cytotoxicity and inhibits glycolysis and migratory capacity in glioblastomas and colorectal cancer cell

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Introduction: The majority of cancer cells present an altered energetic metabolism resorting to aerobic glycolysis, even under aerobic conditions ("Warburg" effect). 3-bromopyruvate (3-BP) is an alkylating agent putatively transported by the monocarboxylate transporters (MCTs), which targets cancer cell metabolism, and it has been demonstrated to be a powerful and specific antitumor agent either in in vitro or in vivo models. 3-BP inhibits tumor energetic metabolism, causing depletion of intracellular ATP, thus acting as a cytotoxic agent. We investigated the effect of 3-BP in glioblastomas, a very aggressive cancer, and in colorectal cancer cell lines, which appears in top three ranking of the most frequent cancer types. 3-BP cytotoxic effect was assessed at different pH values and correlated with monocarboxylates transporters expression. 3-BP effect on migratory capacity and cell metabolism in the different cell lines was also determined. Materials and methods: In the present study, we evaluated the effect of 3-BP in cell viability (using SRB assay) at basal conditions and different extracellular pH (pHe) in glioblastomas (U373MG, U87MG and U251MG) and colorectal (HCT-15, HT-29 and Caco-2) cancer cell lines. In addition, we assessed the migratory capacity (using wound healing assay) and cell metabolism (measuring lactate and glucose in extracellular medium) in the same cell lines. We exposed the different cell lines at ½ IC50 and IC50 values of 3-BP, using as control untreated cells. MCTs expression was estimated by Western-blot assays. Results and discussion: Our results demonstrated that 3-BP presented cytotoxic effect to all cell lines, but with different degrees, depending of the cell line. The cytotoxic effect of 3-BP was higher at pHe 6.6 (pH value usually found in tumor microenvironment) than at pHe 7.4 (physiological pH), consistent with its transport by the MCTs by a proton-symport mechanism. In glioblastomas, 3-BP cytotoxic effect seems to be more associated with MCT4 expression than with MCT1, but in colorectal cancer cell lines no direct association was found. Additionally, we demonstrated that the migratory capacity as

well as energetic metabolism were inhibited by 3-BP, being this effect more evident when higher concentrations of the compound were used.

No conflict of interest

A7. VEGFR2 signaling regulates chemotherapy resistance through reprogramming of mitochondrial metabolism in Acute Myeloid Leukemia

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Metabolic reprogramming plays a pivotal role in cancer progression. However, it is unclear how selective pressures imposed on cancer cells impact on the metabolic-adapted features that contribute for tumorigenesis. We have established a xenograft model of human Acute Myeloid Leukemia (AML), based in the intra-bone marrow injection of human erythroleukemia (HEL) cells on sublethally irradiated immunodeficient Rag2-/-gchain-/- mice, which suitably reproduce AML growth, spreading and associated mortality. In this model, we applied a chemotherapy protocol to induce disease remissions, followed by predictable recurrences of AML and tumor cell variants from mice subjected (chemoHEL) or not (untreatedHEL) to chemotherapy were isolated and established in vitro. Metabolomic analysis by 1H nuclear magnetic resonance revealed that chemoHEL and untreatedHEL variants were metabolically different with increased levels of choline, pyruvate, myo-inositol and saturated fatty acid residues present in the chemoHEL variants. Further metabolic characterization using standard techniques confirmed the presence of higher levels of free fatty acids and lipid droplets in the chemoHEL variants. In addition, we observed that chemoHEL variants had decreased mitochondrial DNA content and mitochondrial mass and higher lactate production, suggesting a strong reliance on glycolysis and a decreased dependence on mitochondrial respiration for cellular energetics. We have previously shown that VEGF receptor signaling is associated with proliferation, survival; increase aggressiveness and chemo-resistance in AML. In addition, VEGF signaling has been recently proposed to regulate anabolic metabolism and growth through FOXO1 in endothelial cells. We observed that chemoHEL variants express higher levels of several chemokine/growth factor receptors, including VEGFR-2 and an increase proportion of VEGFR2 high expressing cells. Moreover, we show that blocking VEGFR2 signaling in vitro with cell-permeable inhibitors sensitizes chemoHEL variants to apoptosis by chemotherapy as measured by AnnexinV+; 7AAD+ staining using flow citometry. Importantly, VEGFR2 inhibition increases the mitochondrial number and reactive oxygen species in chemoHEL variants providing a rationale for the increase sensitization to cell death in the chemotherapy treatment. Overall, our data suggests that VEGFR2 signaling controls the acquisition of chemotherapy resistance through reprogramming of mitochondrial metabolism in AML.

No conflict of interest

A8. The role of MEK5/ERK5 signalling in colon cancer stem-like cells

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Introduction: Cancer stem cells are currently viewed as the reservoir of cells responsible for tumour development, metastasis and drug resistance. However, the molecular players underlying cancer stem cell self-renewal and chemoresistance remain poorly understood. We have previously demonstrated that the MEK5/ERK5 signalling pathway promotes colon cancer metastasis, and that ERK5-targeted inhibition enhances the anticancer properties of 5-fluorouracil in a murine xenograft model. In the present study, we aim to understand the contribution of MEK5/ERK5 signalling for the regulation of the balance between self-renewal and differentiation in colon cancer stem cells Materials and Methods: The effect of MEK5/ERK5 signalling

inhibition was investigated in human colon cancer stem-like cells using both genetic and pharmacological approaches. For this purpose, HCT116, HT29, SW480 and SW620 colon cancer cells were cultured for three generations in sphere-forming conditions. The number of tumour spheres and cells per sphere was determined for each generation. Additionally, the levels of CD44/CD133 expression and aldehyde dehydrogenase 1 (ALDH1) activity were evaluated by flow cytometry. Results and Discussion: Our results demonstrate that tumour spheres enriched in colon cancer stem-like cells have significantly higher levels of MEK5 and ERK5 phosphorylation when compared with their adherent counterparts. In turn, ERK5 inhibition using the pharmacological inhibitor XMD8-92 was shown to decrease the number and size of spheres in HCT116, HT29, SW480 and SW620 cells over three generations. Similarly, ERK5 inhibition using a dominant negative form of MEK5 was shown to decrease HCT116 sphere-forming ability, significantly reducing the number of cells per tumour sphere in third generation spheres, as compared to control spheres. Finally, ERK5 pharmacological inhibition significantly reduced the proportion of CD44/CD133-positive cells, as well as the percentage of cells with high ALDH1 activity. Collectively, our results indicate that MEK5/ERK5 pathway activation may contribute to sustained stemness in colon cancer cells, suggesting ERK5 inhibition as a potential therapeutic strategy to target colon cancer stem cells and improve colon cancer treatment. Supported by UID/DTP/04138/2013, SFRH/BD/96517/2013 and SFRH/BD/88619/2012 from FCT, Portugal.

No conflict of interest

A9. Contribution of miRNA-145 to colon cancer stem cell-like properties

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Introduction A specific subset of cells commonly referred to as cancer stem cells (CSCs), which possess stem cell-like features, is thought to be responsible for tumour initiation and maintenance. miRNAs have recently emerged as promising candidates to target CSCs. miR-145 is a tumour suppressor miRNA, downregulated in colon cancer adenomas and carcinomas. This miRNA has been shown to be involved in tumour growth, metastasis and resistance to chemo/targeted agents, together with modulation of CSC-like properties in prostate cancer and lung adenocarcinoma. In this context, we hypothesise that miR-145 plays a role in the ability of colon CSCs (CCSCs) to self-renew and differentiate. For that, we aimed to evaluate the effect of miR-145 overexpression in maintaining CCSCs-like properties. MATERIALS AND METHODS: We produced miR-145 overexpressing and empty vector control cells in HCT116, HT29, SW480 and SW620 colon cancer cell lines, and examined ability to form colon spheres in ultralow-attachment plates and specific CCSC media. Colon spheres were dissociated to single cells and reseeded to yield the second and third generation of colon spheres. The number of spheres and cells per sphere were counted over 3 generations. CD44 and CD133 expression levels and aldehyde dehydrogenase 1 (ALDH1) activity were evaluated by flow cytometry. RESULTS AND DISCUSSION: Our results showed that forced miR-145 expression had an impact on HT29 and SW620 sphere formation, reducing the number of colon spheres. Moreover, miR-145 overexpression significantly reduced colon sphere diameter and number of cells per sphere in HCT116, HT29, SW480 and SW620 cells. Similar results were observed with the second and third generation of cell line-derived colon spheres. In addition, miR-145 overexpression significantly decreased the proportion of CD44/CD133+ cells and ALDH1 activity (p<0.05). KLF4 mRNA levels were significantly reduced in colon spheres overexpressing miR-145 (p<0.05). The mature colonocyte marker, CK20, was increased in HCT116 spheres overexpressing miR-145 (p<0.01). Collectively, miR-145 appears to be involved in colon sphere formation, self-renewal of colon spheres and differentiation ability of HCT116 colon spheres. miR-145 may contribute to the induction of CCSC differentiation to cells that are sensitive to chemotherapy and targeted agents. Supported, in part, by UID/DTP/04138/2013, SFRH/BD/88619/2012 and SFRH/BD/96517/2013 from FCT, Portugal.

No conflict of interest

A10. The actin cytoskeleton: a key mediator of pre-malignant breast cancer expansion

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Introduction: Tumourigenesis is a multistep process, in which several alterations in oncogenes and tumour suppressor genes condemn normal cells to sustain proliferative signalling, evade growth suppressors, resist cell death and finally invade distant tissues. The c-Src non-receptor tyrosine kinase is one of the oldest and most investigated proto-oncogenes, being overexpressed and/or over-activated in several human cancers, including those of the breast. Basal Src activity, which occurs early during tumor progression, is believed to sustain proliferative signaling and survival of pre-malignant cells. Later, further Src activation may facilitate cell migration, adhesion and invasion of malignant cells via actin filament (F-actin) regulation. Our observations in Drosophila epithelia argue that Src also sustains proliferative signaling and survival of pre-malignant cells via the control of F-actin. Materials and Methods To evaluate the role of F-actin dynamics in the acquisition of the pre-malignant phenotypes by Src, I searched for Actin-Binding Proteins (ABPs) differentially expressed during cellular transformation of the Tamoxifen (TAM)-inducible MCF10A-ER-Src cell line (ER-Src), which recapitulates the natural history of Src-induced breast cancer, and in pre-malignant Estrogen positive (ER+) breast tumour samples. Additionally, we investigated the effect of knocking down or overexpressing each Drosophila orthologs of our candidate ABPs in Drosophila epithelia that contained higher Src levels. Finally, we studied the role of one of the candidates in malignant transformation, using imaging and biochemical approaches and functional assays. Results and Discussion I identified 6 ABPs that are dysregulated in the same direction in both and affect the ability of Src to promote tissue overgrowth in Drosophila epithelia. Among those, the Ena/VASP family member EVL, which promotes the elongation of actin filaments bundles, accumulates upon Src activation. Strikingly, higher EVL protein levels are associated to the transient accumulation of longitudinal actin fibers, an increase in F-actin polymerization and in cellular stiffness. Taken together, my findings argue that during the first phase of Src-induced cellular transformation, EVL builds transient longitudinal actin fibers. In turn, these actin fibers may increase cellular stiffness, which consequently sustains self-sufficiency in growth signals, insensitivity to growth inhibitory signals and evasion of apoptosis.

No conflict of interest

A11. Pharmacological ascorbic acid chemosensitizes colorectal cancer cells and synergistically inhibits tumor growth

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Introduction: Colorectal cancer (CRC) is one of the most dangerous forms of cancer. Despite toxicity caused by conventional chemotherapy, namely 5-flourouracil (5-FU), oxaliplatin (Oxa) and irinotecan (Iri), these are currently used as first and second-line treatment for CRC. Several studies revealed that ascorbic acid (AA) at pharmacological concentrations can act as a pro-oxidant, promoting the formation of reactive oxygen species, such as hydrogen peroxide, which compromise cell viability. A positive feedback has been described in turn of the use of high doses of AA with reduced doses of chemotherapy. So, the aim of this study is to evaluate in vitro and in vivo the therapeutic potential of the combination of AA and conventional chemotherapeutic agents

in CRC. Methods: C2BBe1, LS1034 and WiDr cells were incubated with increasing concentrations of AA and 5-FU, Oxa or Iri, in monotherapy and in combination. Cell proliferation was evaluated through SRB assay after 24, 48, 72 and 96 hours of exposure. The half maximal inhibitory concentrations (IC50) and the combination index were determined. Flow cytometry allowed to evaluate the influence of the treatment on cell viability and the induced types of cell death. For in vivo studies, WiDr cells were inoculated on the back of Balb/c nu/nu mice. AA and the three chemotherapeutic agents were intraperitoneal injected separately or in combination. During 14 days, body weight and tumor size were monitored. Results: In all cell lines, it was observed that when AA concentration increases, cell proliferation decreases, being C2BBe1 cells the most sensitive to AA. In general, IC50 values of all chemotherapeutic agents significantly decreased when present in combination with AA, compared to monotherapy. However, the most promising results were obtained with AA and Oxa combination, being obtained a synergistic effect for all cell lines at 48h and 96h. Combined therapies also caused a decrease on cell viability and, consequently, cell death by apoptosis/necrosis increased. Moreover, the combination of AA and oxaliplatin or AA and irinotecan synergistically inhibited tumor growth. Discussion: Our study suggests that high doses of AA enhance chemosensivity of CRC, even in the multidrug resistant cell line, LS1034. A synergistic effect between AA and conventional chemotherapeutic agents was also observed in a mice model of CRC. The data obtained could contribute to the development of a promising therapy for CRC with reduced doses of conventional chemotherapeutic drugs and consequently, a decrease in secondary effects. The authors would like to thank Foundation for Science and Technology (FCT) (Strategic Project CNC.IBILI: UID/NEU/04539/2013), COMPETE-FEDER for financial support.

No conflict of interest

A12. S100P is a molecular determinant of E-cadherin function in gastric cancer

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Gastric cancer (GC) remains a major concern as the second leading cause of cancer-related mortality worldwide. Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome caused by germline mutations in the E-cadherin encoding gene, CDH1 (Guilford, 1998; van Roy and Berx, 2008). What is striking is that CDH1 germline mutations confer more than 80% lifetime risk to specifically develop gastric cancer, despite that E-cadherin is expressed in all epithelial tissues (Carneiro, 2012). We hypothesize that the gastric epithelium encompasses a specific molecular program allowing CDH1 biallelic inactivation. By evaluating the expression profile of a series of candidate stomach-specific genes, we have identified S100P as a pro-survival factor in gastric cells with loss of functional E-cadherin. S100P inhibition leads to increased cell death in E-cad- gastric epithelial cells, however it does not affect viability nor apoptosis levels in Ecad+ gastric cancer cells. Further, S100P downregulation in E-cad+ cells leads to an increase in aggressive behaviour, inducing a significant increase in the cells´ invasive potential, a significant decrease in E-cadherin expression and an increase in stem cell activity. This data suggests that S100P may be a molecular determinant of E-cadherin function in gastric cancer.

No conflict of interest

A13. The prognostic impact of TERT promoter mutations in glioblastomas is modified by the rs2853669 single nucleotide polymorphism

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Introduction: Human hotspot TERT promoter (TERTp) mutations have been reported in a wide range of tumours. Several studies have shown that TERTp mutations are associated with clinico-pathological features; in some instances, TERTp mutations were considered as biomarkers of poor prognosis. The rs2853669 SNP, located in the TERT promoter region, was reported to modulate the increased TERT expression levels induced by the recurrent somatic mutations. Matherial and methods Representative formalin-fixed paraffin-embedded (FFPE) samples from 504 gliomas were retrieved from the pathology archives of Portuguese institutions (Centro Hospitalar São João, Hospital Pedro Hispano and Hospital de Braga) and Brazilian hospitals (Hospital de Câncer de Barretos and Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto). DNA from FFPE tissues was retrieved from 10μm cuts, The c.-146:G>A and c.-124:G>A hotspot TERTp mutations were screened by PCR followed by direct Sanger sequencing. The sequencing reaction was performed in forward direction. For genotyping the rs2853669 SNP, the electropherogram analysis of the same PCR product allowed the genotyping of rs2853669 SNP. Additionally, in a subset of GBM (n=85) we also used the TaqMan SNP genotyping assay ID: C_8773290_10 (Life Technologies, Carlsbad, USA). The procedure was performed by real time PCR according to manufacturer's instructions in an ABI Prism 7500 Fast system (Life Technologies, Carlsbad, USA). Results and Discussion: TERTp mutations were detected in 47.8% of gliomas (216/452). Glioblastomas (GBM) exhibited the highest frequency of TERTp mutations (66.9%); in this glioma subtype, we found a significant association between TERTp mutations and poor prognosis, regardless of the population. Moreover, in a multivariate analysis, TERTp mutations were the only independent prognostic factor. Our data also showed that the poor prognosis conferred by TERTp mutations was restricted to GBM patients carrying the rs2853669 A allele, and not in those carrying the G allele. In conclusion, the presence of TERTp mutations was associated with worse prognosis in GBM patients, although such association depended on the status of thers2853669 SNP. The status of the rs2853669 SNP should be taken in consideration when assessing the prognostic value of TERTp mutations in GBM patients. TERTp mutations and the rs2853669 SNP can be used in the future as biomarkers of glioma prognosis.

No conflict of interest

A14. Tal1-regulated MIR-146B has a tumor suppressor role in T-Cell acute lymphoblastic leukemia

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Introduction: TAL1, a transcription factor critical for early hematopoiesis, is downregulated early in T-cell development and overexpressed in more than 60% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) cases. Previously we showed that TAL1 regulates microRNA genes at the level of transcription, including miR-146b-5p. Here, we evaluated the functional and molecular effects of miR-146b-5p regulation by TAL1 in the context of T-ALL. Methods: The expression of miR-146b-5p in T-ALL cell lines was determined by real time-PCR whereas in patients and normal counterparts it was analyzed from publicly available data. MiR-146b was manipulated in T-ALL cells using lentiviral transduction and functional impact on proliferation, viability and migration/invasion evaluated by cell counts, flow cytometry and transwell assays, respectively. Effects on motility were further evaluated by immunofluorescence of F-actin distribution and time-lapse confocal

microscopy of cell movements. For in vivo experiments, age-matched NOD/SCID mice were either injected with T-ALL cells overexpressing (CCRF-CEM) or downregulating (MOLT-4) miR-146b-5p and corresponding mock transduced cells. For the analysis of mouse overall survival humane endpoints were established and used to build Kaplan-Meyer curves, tested for significance by using Log-Rank test. Presence of human T-ALL cells was determined by flow cytometry analysis of RFP-positive cells. Results and Discussion: We show that expression of miR-146b-5p is lower in primary T-ALL cells than normal T-cells, thymocytes and other hematopoietic progenitors. MiR-146b-5p silencing enhances the migration and invasion of T-ALL cells, associated with increased levels of filamentous actin and chemokinesis. Consistent with a putative tumor suppressor role, miR-146b overexpression in a TAL1-positive cell line extends mouse survival in a xenotransplant model of human leukemia. In contrast, miR-146b-5p inhibition in results in leukemia acceleration and decreased mouse overall survival, paralleled by faster tumor infiltration of the central nervous system. Our results suggest that miR-146b-5p is a functionally relevant TAL1 microRNA target gene which may act as tumor suppressor in developing T-cells by modulating cell mobility, migration and invasion in vitro and decreasing T-ALL aggressiveness in vivo.

No conflict of interest

A15. Centrosome Amplification in Human Cancerigenesis: where, when and how?

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Introduction: Centrosome abnormalities are a typical hallmark of human cancers. As the major microtubule-organizing centre in animal cells, the centrosome has key roles in signaling, polarity and bipolar mitotic spindle formation. Deviation from normal centrosome numbers can lead to chromosome segregation errors and altered invasive and migratory behavior in cultured cells. As centrosome abnormalities are found in many human tumors but not normal cells, they represent an appealing feature for diagnosis, prognosis and therapy. However, despite the accumulating evidence supporting the hypothesis that centrosomes are more than simple bystanders in tumor progression, crucial questions about their origin in vivo remain unanswered. Here, we used a human cancer model with a well-defined pathway of progression to determine when and how centrosome number amplification arises in human tumor initiation and progression. Materials and Methods We established a new method to unequivocally identify and examine bona fide centrosomes in paraffin-embedded clinical samples at the single cell level, and applied it to a clinically well-established and genetically well-defined pathway of cancer progression that allows the unique study of all steps of malignant transformation in each individual patient: Barrett's esophagus cancerigenesis; a multistep process from metaplasia (the premalignant condition) to dysplasia (non-invasive intra-epithelial neoplasia) and adenocarcinoma (invasive neoplasia). Additionally, we took advantage of a panel of cell lines that represent all stages of disease found in vivo to get further mechanistic insight into how centrosome abnormalities arise. In particular, we assessed the role of p53 tumor suppressor and its hotspot mutations in generating centrosome number abnormalities. Results and Discussion We found that centrosome number amplification is present as early as the premalignant condition and that its incidence changes throughout malignant transformation, being significantly expanded in dysplasia. We show that this early centrosome amplification correlates with and is dependent upon loss-of-function of the tumor suppressor p53. Our work defines the timing, dynamics and mechanistic regulation of centrosome amplification. Given the widespread occurrence of both p53 mutations and centrosome amplification in human tumors, our findings are likely to be extended to other cancers.

No conflict of interest

A16. A model to study cancer stem cell metabolic changes in glioblastomas

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Cancer stem cells are a specific pool of cells that are thought to be responsible for cancer growth and recurrence. Glioblastomas (GB) are the most lethal primary brain tumours, showing a high rate of recurrence. GBs are heterogeneous tumours that contain neoplastic stem-like cells, named glioblastoma stem cells (GSCs), which have been associated with therapy resistance and tumour recurrence in a c-Met dependent pathway. Thus, GB seems to be a particularly relevant model to decipher the mechanisms that govern cancer cell stemness, specifically those related to metabolic networks. Using specific culture conditions - neurosphere formation assays – we have selected cells with anoikis resistance and self-renewal capacities, known as GSCs, from two glioblastoma cell lines (U87 and U251) and a normal human astrocyte cell line (NHA). We are conducting a comparative analysis of stem cells markers (CD133, SOX2, Nestin) and differentiation markers (GFAP) in GSCs and non-GSCs, in order to validate the model. Our results indicate that Nestin is a good marker that distinguishes GSCs from non-GSCs, while CD133 and SOX2 were not detected. Exploratory analyses indicate that the c-Met receptor is differentially phosphorylated in non-GSCs and that, pivotal metabolic enzymes, such as pyruvate dehydrogenase, may play a role in sustaining the GSC phenotype. We expect to reveal the features that may help in defining the metabolic portrait of GSCs.

No conflict of interest

A17. Expression of tumor-related Rac1b antagonizes B-Raf-induced senescence in colorectal cells

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Introduction: Mutations in the BRAF oncogene have been identified as a tumor-initiating genetic event in mainly melanoma, thyroid and colon cancer, resulting in an initial proliferative stimulus that is followed by a growth arrest period known as oncogene-induced senescence (OIS). It remains unknown what triggers subsequent escape from OIS to allow further tumor progression. A previous analysis revealed that around 80% of colorectal tumors carrying a mutation in BRAF also overexpress splice variant Rac1b. Materials and Methods: We used normal NCM460 colonocytes as a model to express oncogenic B-Raf-V600E in the presence or absence of co-transfected Rac1b and then analyzed the effect on expression of senescence markers. Results and Discussion: When oncogenic B-Raf-V600E was expressed we observed the induction of the senescence-associated β-galactosidase and of the cell-cycle inhibitors p14, p15 and p21 whereas proliferation marker Ki67 was suppressed. Upon co-expression of splice variant Rac1b, but not of Rac1, the B-Raf-induced senescence phenotype was reverted and expression of the cell-cycle inhibitors downregulated in a reactive oxygen-species dependent manner. We thus provide evidence that co-expression of splice variant Rac1b counteracts B-Raf-induced senescence, indicating the selection for increased Rac1b expression as one potential mechanism by which colorectal tumor cells can escape from B-Raf-induced OIS.

No conflict of interest

A18. Basal-like breast cancer phenotype: is it necessary during early stages of cancer development?

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Introduction: Basal-like breast cancer is an intrinsic molecular subtype associated to poor patient prognosis, characterized by the overexpression of basal/myoepithelial markers by neoplastic cells, such as CK5, CK17, and P-cadherin. Recently, it has been shown that the acquisition of a basal-like phenotype by breast cancer cells is closely related with their invasive capacity. However, it is not known if this phenotype is also important to be acquired during early stages of breast cancer development. Material and methods TAM-inducible MCF10A-ER-Src normal breast cells was used as a model of breast cancer initiation/transformation. cDNA microarray profile data was analysed in different time points after tamoxifen treatment, to follow the dynamics of basal markers during transformation. siRNA and over-expression strategies for basal markers were tested in 2D and 3D cell cultures, which were followed by time-lapse microscopy, and mammosphere assay. Results and discussion In 2D, morphological cell transformation is only clear at 24h after Src-induction. However, the analysis of RNA transcriptional profile during cellular transformation, revealed that basal markers are induced in early time points of Src-induced transformation. The increase in basal keratins and P-cadherin already occur 8h after the beginning of transformation. Notably, this increase was validated by western blot. Interestingly, EMT markers were not significantly altered during transformation as initially predicted. In 3D matrigel cultures, the induction of Src by tamoxifen causes epithelial acini disorganization, as well as increases colony formation and mammospheres formimg efficiency of MCF10A cells. Importantly, these effects were reverted by P-cadherin silencing. However, its overexpression in non-transformed cells does not induce transformation in 2D or 3D cultures. Although preliminary, these results show that basal markers are possibly needed to be upregulated for the progression of early transformed cells, not acting themselves as triggers of cell transformation. To prove this, these experiments are being now further validated for the silencing/overexpression of other basal markers.

No conflict of interest

A19. Characterization of exosomes isolated from 2D and 3D cell culture: does the third dimension change the exosomal information?

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Introduction: Exosomes are extracellular nanovesicles carrying specific biomolecules such as miRNA, mRNA and proteins. These biologic conveyors act in intercellular communication, being important mediators in several biologic contexts, namely in tumour-related interactions. Despite the vast literature published in this field, no studies have reported the production of exosomes by cells growing in 3D cultures. In fact, 3D models emerge as more realistic and physiological in vitro systems, compared to the traditional 2D monolayers. Our aim was to characterize markers and content of exosomes produced by cancer cells grown in 2D and 3D conditions. Materials and Methods: We characterized exosomes released by 2 gastric and 2 breast cancer cell lines, cultured in 2D and 3D conditions. For 2D conditions, cells were cultured in monolayers; as for 3D cultures, cells were grown as single and independent spheroids. In both conditions, cells were grown in appropriate culture media, supplemented with exosome-depleted FBS, at 37°C in a 5% v/v CO_2 humidified atmosphere. Cellular growth and spheroid formation was followed by optical microscopy, and H&E of spheroids was performed at different time-points. Exosomes were isolated from the conditioned media of 2D and 3D cultures by differential ultracentrifugation and characterized by TEM, Nanosight and imaging flow cytometry for the presence of exosomal markers (e.g. CD9). Exosomal RNA was extracted with miRCURY™ RNA Isolation Kits - Biofluids and quantified in TapeStation 2200, using HS RNA tape. Exosomal protein was quantified using

Pierce™ BCA Protein Assay Kit. Results and Discussion: We verified that exosomes secreted by cells cultured in 2D and 3D display similar sizes (50-150nm) and express the exosomal markers CD9, CD81 and flotillin. We also found that cells grown in 3D spheroids secrete more exosomes than those cultured in 2D monolayers, which correlates with considerable higher amounts of exosomal RNA and protein. GC and BC cell lines display particular abilities to grow in 3D, forming spheroids with variable shapes and structures. Particularly, spheroid growth was dependent on cell density at the moment of seeding, and on FBS supplementation. Comprehensive comparative proteomics and miRNA profiling of exosomes isolated from 2D and 3D conditions will be performed to estimate the impact of growth conditions on exosome content. This work is funded by: 1) FEDER/COMPETE, FCT/MEC/FEDER/PT2020 and FCT funds (projects "PEst-C/SAU/LA0003/2013"; project 007274 (UID/BIM/04293); 2) ON.2–O Novo Norte/FEDER/QREN (projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067); 3) FCT PhD Programmes and Programa Operacional Potencial Humano (POCH), specifically by the BiotechHealth Programe (Doctoral Programme on Cellular and Molecular Biotechnology Applied to Health Sciences); 4) FCT Fellowships (SFRH/BPD/86543/2012 to JC; PD/BI/113971/2015 to SR).

No conflict of interest

A20. CD44v6 containing transcripts influence drug response in gastric cancer cells

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Introduction: CD44 is the principal cell surface receptor for hyaluronic acid and has been associated with tumorigenesis and metastasis. Nonetheless, the relationship between CD44 protein expression and clinical-pathological features of gastric cancer (GC), its role as a prognostic marker remains controversial. Recently, we identified overexpression of CD44v6 in >60% of GC, however its expression was also present in a high frequency in gastric pre-malignant lesions. These data questioned the role of CD44 as part of the initiation signature of GC. However, given the role of this particular transcript in cancer stemness, we hypothesized that CD44v6 overexpression could condition the response to conventional chemotherapy generally used in GC patients. Materials and Methods: We used an isogenic GC cell line model either lacking CD44 expression (Mock) or overexpressing specifically CD44v6 or the CD44std, as well as cells lines endogenously expressing CD44-containing transcripts. We also depleted CD44v6 in cells endogenously expressing this molecule by RNAi with siRNAs. We tested all cell lines for their response to the conventional chemotherapeutic drug cisplatin, using cell growth/survival (resazurin-based and SRB assays) and apoptosis detection assays by flow cytometry. We assessed the expression of a downstream effector of CD44, p38, in response to cisplatin treatment, by Western blot and immunocytochemistry. Results and discussion: Induced and endogenous expression of CD44v6 increased the resistance to cisplatin treatment. Most importantly, this effect was counteracted when CD44v6 is depleted in several cell lines. Total expression of p38 did not change in response to cisplatin treatments. However, p38 was found more frequently localized in the nucleus of CD44v6 overexpressing cells, suggesting an axis linking CD44v6 with p38 in cisplatin-resistance. Our results suggest that depletion of CD44v6 may sensitize GC cells for cisplatin-based chemotherapy regimens. Support: FEDER/COMPETE/ FCT projects: PEst-C/SAU/LA0003/2013; FCT/MEC/PT2020 nº007274-UID/BIM/04293); FCT project PTDC/CTM-NAN/120958/2010; ON.2/QREN projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067; FCT Fellowships (PD/BD/105976/2014 to DF and SFRH/BD/113031/2015 to CP) and Salary support to GMA from the iFCT Program 2013 (IF/00615/2013), POPH - QREN Type 4.2, ESF and MCTES.

No conflict of interest

A21. Wireless communication in pancreatic cancer: exosomes mediate intratumoral network

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Introduction: Exosomes are extracellular vesicles that mediate intercellular communication with neighbor and distant cells and their cargo alters function and physiology of recipient cells. Pancreatic cancers are known for their heterogeneity and for the presence of hierarchical cancer cell subpopulations, which cooperate to drive and maintain tumor progression. If and how exosomes are involved in this intra-tumor communication is not known. Exosomes have been shown to contribute for both horizontal reprogramming and re-education of recipient cells. Therefore, we hypothesized that exosomes from different subpopulations transfer information that modulates the cooperation between cancer cells and enhance tumor plasticity. Materials and Methods: Based on surface markers, subpopulations of pancreatic cancer cells were identified using fluorescent activated cell sorting. Exosomes isolated from each subpopulation were characterized by nanoparticle tracking analysis and transmission electron microscopy. Exosomal protein and transcripts content was analyzed by Mass Spectroscopy and RNA Sequencing. Stable clones combining known exosomes markers with a fluorescent protein, secreting color-coded exosomes, were stablished and used in co-cultures to study the exosomes flow between the different subpopulations. The co-cultures were analyzed by advanced optical imaging and flow cytometry. Results and discussion: We have identified five subpopulations of pancreatic cancer cells with exosomes showing expression for CD63, CD9, CD81, CD82 and Rab5 exosomal markers. No significant differences in the size of the exosomes were observed between these subpopulations. Co-cultures demonstrated the presence of multicolor positive cells, which indicates that exosomes are exchanged between different subpopulations. Hence, revealing a putative dynamic network of communication, mediated by exosomes, among phenotypically different cancer cells. In order to understand the role that exosomes content might have in the receiving cells, we characterized the proteome and transcriptome of exosomes released by each subpopulation of cancer cells. Interestingly, subpopulations of pancreatic cancer cells, which have cancer-stem cell-like phenotype produce more exosomes per cell and those exosomes contain more proteins which are specific of the exosomes released by that specific subpopulation, not being found in other exosomes released by other subpopulations of pancreatic cancer cells. Therefore, the communication network mediated by exosomes among well defined subpopulations of pancreatic cancer cells might be involved in tumor progression and metastatic disease.

No conflict of interest

A22. Mutant kras mediates fibroblast-induced colorectal cancer cell invasion

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Introduction: Colorectal cancer (CRC) is a very frequent type of cancer, being responsible for numerous deaths worldwide. CRC tumorigenesis is the result of a stepwise accumulation of mutations, with KRAS mutations occurring early in this process. Mutations in this oncogene account for almost 40-50% of the cases of CRC and have been implicated in cancer cell proliferation, survival, migration, invasion, loss of differentiation and stemness. However, cancer cells do not exist as isolated entities. In fact, tumors have increasingly been recognized as a complex and heterogeneous network with tumor cells communicating with each other and with the surrounding stromal components, creating a pro-tumorigenic microenvironment that is determinant for progression. We hypothesize that KRAS effects could extend beyond the tumor cell itself, impacting cell-cell and cell-matrix communication. Therefore, in this work we aim to: (i) investigate whether KRAS activation in

cancer cells may affect their response to extracellular stimuli; (ii) identify the molecular events underlying this effect. Materials and Methods: In order to evaluate the effect of KRAS activation in the response of CRC cells to external stimuli, we performed matrigel invasion assays with a KRAS mutant CRC cell line, HCT116, and using fibroblasts' conditioned media (CM) as a chemoattractant. Additionally, resorting to western blot, immunofluorescence and flow cytometry, we evaluated the expression several cell surface proteins. Results and Discussion: Our results show that HCT116 do not invade the matrigel layer when treated with control CM but become invasive in response to fibroblasts' CM. However, inhibition of KRAS by siRNA impairs HCT116 invasion. These results suggest that KRAS may be an important modulator of response to fibroblasts' secreted factors that induce CRC cells invasion. To better understand how KRAS exerts this effect, we evaluated the expression of several cell surface proteins involved in cell-cell communication and cell-matrix adhesion. We observed that KRAS regulates the expression levels of c-MET, CD49f and β4 integrins, CD44, and E-Cadherin. In brief, our data suggests that mutant KRAS plays an important role in fibroblasts-induced CRC cell invasion, possible through the regulation of numerous cell surface proteins and associated downstream signaling.

No conflict of interest

A23. Modulation of colorectal cancer cell differentiation markers by oncogenic alterations

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Introduction: Colorectal cancer (CRC) is one of the most common cancers worldwide and mutations regarding KRas, BRaf and PIK3CA oncogenes are usually present in more than a half of the cases. Recent studies indicate that KRas mutations in CRC patients with APC loss background enhanced cancer stem cells (CSCs) activation, therefore tumour growth. Given the importance of CSCs for tumorigenesis, recurrence, metastasis and therapy resistance, in this work we aimed at investigate the role of these oncogenic alterations in modulating CRC cell differentiation and ultimately induction of CSC properties. Materials and Methods We have characterized several stem cell/differentiation markers through flow cytometry and real time PCR, after inhibition of KRas, BRaf and PIK3CA in HCT-116, SW480, HCT-15 (mutated KRas and PIK3CA) and RKO (mutated BRaf and PIK3CA) CRC cell lines. Results and Discussion Our work shows that inhibition of mutant KRas decreases the number of CD44 positive cells in HCT116 cell line, and induces a shift of the remaining positive pool towards a CD44low population. Furthermore, significant but low changes were observed on CD133 positive cells, while CD166 had no significant variation. Moreover, BRaf inhibition slightly decreases the number of CD44 positive cells in RKO cell line, and CD133 positive cells in HCT116 cell line. No significant alterations were observed when PIK3CA was inhibited. To confirm if alterations on cancer stem cell markers might result on alteration of stemness, colosphere-forming assay was performed. Using KRas inhibited HCT116 cell line, we observed a reduction in the number of colospheres formed, which although not significant, shows a tendency towards a less stem phenotype. We have also evaluated the expression of the intestinal stem cell (ISC) markers LRIG1 (a marker for quiescent ISC) and LGR5 (promotes adhesion and reduces clonogenicity of CRC cells). The expression of both markers was increased upon KRas inhibition in HCT116 cell line. Accordingly, our data suggests that mutant KRas and BRaf may have overlapping effects on the induction of CSC properties through modulation of CD44 and CD133 expression whereas PIK3CA does not show the same effect. Moreover, the results also suggest that KRas may function as a regulator of LRIG1 and LGR5 expression which, according to recent data suggesting a tumour suppressor role for these ISC markers, may be associated with a decrease in CRC cell proliferation, motility and invasion.

No conflict of interest

A24. SDHD promoter mutations and SDHD protein expression in cutaneous melanoma

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Introduction: SDHD is one of the four Succinate Dehydrogenase (SDH) complex subunits. SDHD promoter mutations were recently reported in 10% of cutaneous melanoma (CM), based on data mining, and associated with decreased gene expression and poor prognosis. Then, 4% of SDHD promoter mutations were reported in a cohort of cutaneous melanoma samples, although not related with clinico-pathological factors or patient survival. This study aimed to verify the presence of SDHD promoter mutations and SDHD protein expression in our melanoma series and the possible association with prognosis and survival of the melanoma patients. Material and Methods: We assessed the SDHD promoter status in melanoma cell lines (n=4), nevus (n=5) and in CM (n=86), previously evaluated for BRAF, NRAS and TERT promoter mutational status. We also examined the expression of SDHD protein by immunohistochemistry in 113 CM. In addition, we searched the putative association between the presence of SDHD protein expression and the clinico-pathological and prognostic parameters of CM. Results and Discussion: We detected 2% (two cases) of SDHD promoter mutations in CM, but none in nevus and cell lines. One positive case (superficial spreading melanoma) did not displayed BRAF, NRAS and TERT mutations, and the patient was alive at the last follow-up (180 months). The other positive case (acral melanoma), displayed TERT promoter mutation, but not BRAF mutation; the patient died 24 months after diagnosis, in line with the poor prognosis of CM harbouring TERT promoter mutations. SDHD protein expression was present in most CM (86%), including those with SDHD promoter mutations. Due to the lower number of mutated cases, we cannot infer if there is a reduction in the protein expression level caused by the presence of SDHD promoter mutation or if there is any association between this mutation and the clinico-pathological and prognostic parameters in CM. However, we found a significant association between the lower mean SDHD protein expression and the presence of ulceration and higher TNM stages, but not with disease free and overall survival of the patients. Our results suggest that SDHD promoter mutation is a rare event in CM, but SDHD lower expression (not related with the presence of the mutation) might be associated with worst prognosis features of CM.

No conflict of interest

A25. Targeting Spindly as Antimitotic Strategy to Induce Cancer Cell Death

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Introduction: Chemotherapy represents the most common therapeutic approach against a broad range of solid cancers, with Paclitaxel (Taxol) being one of the most potent and effective anti-neoplastic drugs for some of these cancers. Belonging to the family of the anti-microtubules agents, Taxol targets microtubules dynamic, induces prolonged mitotic arrest dependent on spindle assembly checkpoint (SAC) activity and triggers cell death [1, 2]. However, the development of chemoresistance remains a limitation for its clinical success in cancer therapy. A great challenge is the development of new therapeutic strategies, alone or in combination, to kill cancer cells or enhance their response to chemotherapeutics drugs. Spindly is a mitotic protein with a key role for accurate chromosome congression and segregation during mitosis. Spindly depletion mediated by

RNAi has been shown to induce a severe chromosome misalignment phenotype and block mitotic progression in a SAC-dependent manner [3], a behavior that mirrors Taxol treated-cells. This observation prompted us to explore the role of Spindly as a potential therapeutic target for cancer treatment, either individually or in combination with Taxol. Materials and Methods Spindly expression was assessed by quantitative real-time PCR in human brain cancer cell lines. Spindly expression was down-regulated with small interfering RNAs (siRNAs). Time-lapse microscopy was performed to follow cell fate. To assess the effect of Spindly inhibition, either alone or in combination with Taxol, we focused on cell viability and proliferation, by the MTT cytotoxic assay and clonogenic assay, respectively. Cell cycle distribution profile and apoptosis were analyzed by flow cytometry. Results and Discussion Spindly was found overexpressed in brain cancer cell lines. Following siRNA-mediated Spindly inhibition, cancer cells were blocked in mitosis followed by apoptotic cell death, in a caspase-dependent pathway. Suppression of Spindly compromised cell viability and caused enhancement of Taxol's cytotoxicity (at clinically relevant doses). This was particularly evident in a 10 days clonogenic assay. Our results show, for the first time, that selective inhibition of Spindly induces cancer cell death and could potentiate the effect of Taxol. Therefore, Spindly may represent a potential therapeutic target for cancer therapy. [1] Schiff PB, Fant J, Horwitz SB. Nature. 1979;277(5698):665-7. [2] Sena G, Onado C, Cappella P Et al. Cytometry. 1999;37(2):113-24. [3] Barisic M, Sohm B, Mikolcevic P et al. Mol Biol Cell. 2010;21(12):1968-81.

No conflict of interest

A26. Role of the androgen-target gene regucalcin in breast and prostate physiology

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Introduction: Regucalcin (RGN) is a calcium (Ca2+)-binding protein that, besides participating in intracellular Ca2+-homeostasis, seems to regulate cell proliferation and apoptosis. RGN was previously found to be downregulated in breast and prostate cancer cases. Androgens actions are well-established in the context of prostate physiology, but these hormones also seem to influence mammary gland function. Although it has been shown that androgens regulate RGN expression in LNCaP cells, their effects in vivo are unknown. This work evaluated the effect of 5α-dihydrotestosterone (DHT) in RGN expression in rat prostate and MCF-7 cells, and investigated the RGN functions in breast and prostate. Material and Methods: Transgenic rats overexpressing RGN (Tg-RGN) and controls were used to evaluate the effect of RGN in modulating cell proliferation/apoptosis. Quantitative real-time PCR (qPCR), Western blot (WB) and enzymatic activity were used to determine the expression (activity) of cell cycle and apoptosis regulators. Proliferation was estimated using Ki67 immunohistochemistry. qPCR and WB were also used to assess the effect of DHT in RGN expression in rat prostate using control, orchidectomized (ORCHX), and ORCHX plus DHT treated rats. RGN expression was also determined in MCF-7 cells cultured in the presence of 1nM DHT or 1nM DHT plus 1μM of flutamide. MCF-7 cell viability was evaluated by the MTS assay. Results and discussion: Overexpression of RGN diminished rat prostate cell proliferation (Ki67 index) concomitantly with the downregulation of p21 and HRas. In the mammary gland of Tg-RGN animals, p53 expression was upregulated whereas Myc and Cdk1 gene expression was diminished. RGN supressed apoptosis in the prostate of Tg-RGN as indicated by the increased Bcl-2/Bax protein ratio and decreased activity of caspase-3; in the mammary gland of transgenic animals, caspase-3 activity was increased. RGN levels increased in ORCHX rats returning to control levels in the ORCHX plus DHT group. The downregulation of RGN expression in response to DHT was also seen in MCF-7 cells, and seems to depend on the androgen receptor since flutamide abolished the effect. DHT also diminished MCF-7 cell viability, with underpinned augmented expression of p53. These results suggest that RGN plays a significant role in breast and prostate tissue homeostasis. Moreover, as an androgen target gene this protein may have an important role in breast and prostate (patho)physiology.

No conflict of interest

A27. NFE2L2 andKEAP1 gene expression in Myelodysplastic Syndrome and Monoclonal Gammopathies

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Oxidative stress (OS) deregulation has been observed is almost all neoplastic disorders, including leukemia, where it contributes to disease development and progression. NRF2 (Nuclear factor erythroid-2-related factor 2) is a transcription factor codified by NFE2L2 gene (Nuclearfactorerythroid2-like 2) that activate many antioxidant and detoxification genes, and is strongly regulated by KEAP1 (Kelch-like ECH-associated protein 1).NRF2-KEAP1 system seems to have an extremely important role in OS regulation, contributing for disease development and/or influence therapy response. Our aim was to evaluate the expression levels of NFE2L2 and KEAP1 genes in Myelodysplastic Syndrome (MDS) and Monoclonal Gammopathies (MG) patients, as well as its correlation with clinical and laboratorial data, in order to identify potential biomarkers of diagnosis and/or prognosis. We evaluated 135 samples, 53 MDS (26 refractory cytopenia with multilineage dysplasia; 8chronic myelomonocytic leukemia; 7 refractory cytopenia with unilineage dysplasia; 5 refractory anemia with excess blasts-1; 4 refractory anemia with ringed sideroblasts; 2refractory anemia with excess blasts-2; 1 5q), 40 MG (15 monoclonal gammopathy of undetermined significance; 25 multiple myeloma) and 42 controls (CTL). Samples were collected after informed consent obtained in accordance with the Helsinki Declaration. Real-time PCR was used to evaluate the gene expression levelsofNFE2L2, KEAP1 and GUS (control gene). Results were considered statistically significant when $p<0,05$. Our results showed that patient's group presented higher expression levels ofKEAP1 gene than control group (Patients: 0,333; CTL: 0,099; p=0,023), while no differences were observed for NFE2L2 gene. In the evaluation by pathology, we were able to observe that KEAP1 gene expression levels was higher in GM patients compared to controls (GM: 0,2141; CTL: 0,0991; p=0,002), but lower when compared with MDS patients (GM:0,2141; MDS: 0,4232; p= 0,03). No association was found between MDS and CTL, however RCMD patients presented lower levels of NFE2L2 (RCMD: 1,665; CTL: 4,142; p=0,0007) and higher level of KEAP1 gene (RCMD: 0,1799; CTL: 0,0991; p=0,005). ROC analysis showed that KEAP1 levels might be diagnostic biomarkers for GM patients (cut off > 0,1931; sensitivity: 45%; specificity: 90,48%), as well as NFE2L2 (cut off < 2,265; sensitivity: 84,62%; specificity: 64,29%) and KEAP1 levels (cut off > 0,1664; sensitivity: 53,85%; specificity; 83,33%) for RCMD. Through survival analysis we observed that RCMD patients with NFE2L2 expression levels under 2,265 present a tendency for lower survival. These results suggest that expression levels of NFE2L2 and KEAP1 genes might be associated with development of hematological malignancies, and may be potential diagnostic biomarkers for Myelodysplastic Syndrome and Monoclonal Gammopathies. This study is supported by Center of Investigation in Environment Genetics and Oncobiology (CIMAGO), Jorge J. by LPCC-NRC/CIMAGO 2015 Grant, Pires A. by LPCC-Pfizer 2015 Grant andAlves R. is supported by Portuguese Foundation to Science (FCT) grant (SFRH/BD/51994/2012).

No conflict of interest

A28. Exploiting the role of androgens as modulators of glutaminolysis in prostate cancer: a new therapeutic approach?

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Introduction: Progression of prostate cancer (PCa) is dependent on androgens, enabling the use of therapies reducing androgens levels or antagonizing the androgen receptor (AR). However, PCa cells tend to proliferate independently of androgen depletion, which leads tumours progressing to a castrate-resistant stage (CRPC). Previous work of our research group has suggested that progression to CRPC is associated with a metabolic adaptation. Moreover, we demonstrated that androgens regulate glycolytic metabolism in PCa cells by modulating the expression of glucose transporters/glycolytic enzymes. However, the effect of androgens in

glutaminolysis is unknown. This study aimed to analyse the expression of glutaminolysis regulators in PCa cells and determine the influence of androgens in glutaminolysis. Methods: The expression of glutamine transporter, ASCT2 and glutaminase in LNCaP, DU145 and PC3 cells was evaluated by Western Blot (WB). Moreover, PCa cells were treated with 10 nM of 5α-dihydrotestosterone (DHT) for 12-48 h. WB analysis was used to determine the effect of DHT on ASCT2 and glutaminase expression levels. The production of glutamine by PCa cells in response to DHT was estimated spectrophotometrically using a commercial kit. Also, the effect of DHT on the subcellular localization of ASCT2 was investigated by means of colocalization with the endoplasmatic reticulum (ER) protein calnexin. The effectiveness of glutaminase and AR inhibition on PCa cell proliferation/viability was evaluated by the MTT assay. Results and Discussion: CRPC cells (DU145 and PC3) displayed decreased expression of ASCT2 and increased expression of glutaminase relatively to LNCaP. DHT treatment for 24 h increased the expression of the stable isoform of ASCT2 in LNCaP cells whereas decreasing the expression of the unstable isoform. Also, glutaminase expression was increased after 24 h of DHT treatment. In addition, DHT stimulated the colocalization of ASCT2 with ER in LNCaP cells. The specific inhibitor of glutaminase BPTES decreased PCa cell viability in a dose-dependent manner. Moreover, BPTES and bicalutamide cotreatment demonstrated to be more effective than single administrations. The present findings showed that the expression of glutaminolysis regulators in PCa is related with the aggressiveness of disease, and regulated by androgens. These results also indicated that the simultaneous inhibition of AR and glutaminase has the potential to be exploited as a new PCa therapy.

No conflict of interest

A29. Insight into the molecular basis of Schistosoma haematobium-induced bladder cancer through urine proteomics

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Introduction: Infection due to Schistosoma haematobium is carcinogenic. However, the cellular and molecular mechanisms underlying urogenital schistosomiasis (UGS)-induced carcinogenesis have not been well defined. Conceptually, early molecular detection of this phenomenon, through noninvasive procedures, seems feasible and is desirable. In order to increase the knowledge on S. haematobium-induced bladder cancer and identify potential biomarkers for its diagnosis, an exploratory proteome profiling of urine from patients with UGS or/and bladder cancer was performed. Materials and methods: Six patients diagnosed with UGS in Angola (three of them also with bladder cancer) and three patients diagnosed with urothelial cancer at IPO-Porto were enrolled in the present study. Urine samples were centrifuged and the supernatant passed through filters to concentrate protein content and remove salts. The final retentate was characterized by GeLC-MS/MS. Results and discussion: Proteome analysis retrieved 535 proteins, from which only 74 were common to the three groups of patients. Seventeen proteins were only identified in the urine of UGS patients, 42 were unique to patients with UGS-induced bladder cancer, 25 were unique to patients with urothelial carcinoma, 27 were common to patients with UGS with and without bladder cancer and 8 were common to patients with bladder cancer, with and without UGS. Protein-protein interaction analysis indicated oxidative stress and immune defense system responsible for microbicide activity as the most representative clusters in UGS patients.

Proteins involved in immunity, negative regulation of endopeptidase activity, and inflammation were found more prevalent in UGS patients with bladder cancer, whereas proteins with roles in renal system process, sensory perception of taste and gas and oxygen transport were more abundant in subjects with urothelial carcinoma not associated with UGS. These findings highlighted a Th2 type immune response induced by S. haematobium, which seems to be further modulated by tumorigenesis, resulting in high-grade bladder cancer characterized by an inflammatory response and complement activation alternative pathway. These findings established a starting point for the development of multimarker strategies for the early detection of UGS-induced bladder cancer. Acknowledgements This work was supported by the Portuguese Foundation for Science and Technology (FCT), European Union, QREN, FEDER and COMPETE for funding the QOPNA, iBiMED research unit (project PEst-C/QUI/UI0062/2013, UID/BIM/04501/2013) and PhD fellowship SFRH/BD/80855/2011 (CB), and by the Portuguese Mass Spectrometry Network (RNEM). The authors also acknowledge Clínica Sagrada Esperança and Serviço de Urologia do Hospital Américo Boavida from Luanda, Angola.

No conflict of interest

A30. Preventing E-cadherin aberrant N-glycosylation at Asn-554 improves its critical function in gastric cancer

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E-cadherin is a central molecule in the process of gastric carcinogenesis and its posttranslational modifications by N-glycosylation have been described to induce a deleterious effect on cell adhesion associated with tumor cell invasion. However, the role that site specific glycosylation of E-cadherin has in its defective function in gastric cancer cells needs to be determined. Using transgenic mice models and human clinical samples, we demonstrated that N-acetylglucosaminyltransferase V (GnT-V)-mediated glycosylation causes an abnormal pattern of E-cadherin expression in the gastric mucosa. In vitro models further indicated that, among the four potential N-glycosylation sites of E-cadherin, Asn-554 is the key site that is selectively modified with β1,6 GlcNAc-branched N-glycans catalyzed by GnT-V. This aberrant glycan modification on this specific asparagine site of E-cadherin was demonstrated to affect its critical functions in gastric cancer cells by affecting E-cadherin cellular localization, cis-dimer formation, molecular assembly and stability of the adherens junctions and cell–cell aggregation, which was further observed in human gastric carcinomas. Interestingly, manipulating this site-specific glycosylation, by preventing Asn-554 from receiving the deleterious branched structures, either by a mutation or by silencing GnT-V, resulted in a protective effect on E-cadherin, precluding its functional dysregulation and contributing to tumor suppression.

No conflict of interest

A31. Evaluation of Glucose and Monocarboxylate transporters and CD147 in papillary renal cell carcinoma.

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Evaluation of Glucose and Monocarboxylate transporters and CD147 in papillary renal cell carcinoma. Introduction: Renal cell carcinomas (RCC) are heterogeneous tumours considered to arise from the epithelium of renal tubules, comprising > 90% adult kidney carcinomas. Clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC), and chromophobe renal cell carcinoma (chRCC) are most common adult RCC. pRCC is classified in two subtypes (pRCC1 and pRCC2) based on distinct morphological and behavioural features which may influence therapeutical options. There is growing evidence that cancer cells display metabolic modifications, and RCC is known by frequent alterations in metabolic proteins. We aimed to evaluate the expression of metabolic markers [Glucose transporters (GLUTs) and Monocarboxylate transporters (MCTs) as well as its chaperon CD147] in pRCC to substantiate if they may help characterization of both pRCC subtypes and their biological behaviour. Material and Methods: Immunohistochemical expression of membrane proteins GLUT1, GLUT4, MCT1, MCT4 and CD147, was assessed in 51 primary pRCC: pRCC1 (n=45; 88.2%) and pRCC2 (n=6; 11.8 %). Results and Discussion: GLUT1, GLUT4, MCT1, MCT4 and CD147 expression was detected in 60.8%, 9.8%, 17.6%, 94.1% and, 60.8% pRCC, respectively. Interestingly, expression of GLUT1 and MCT1 was significantly more frequent in pRCC2: GLUT1 in 100% pRCC2 vs 55.6% pRCC1 (p=0.044); MCT1 in 83.3% pRCC2 vs 8.9% pRCC1 (p<0.001); but no statistically significant differences were detected for the other metabolic marker expression when comparing both pRCC subtypes. Noteworthy, expression of GLUT1 and MCT1 significantly correlate with higher Fuhrman grade (p<0.001 and p=0.033, respectively) of pRCC. Our results show that pRCC display glycolytic features, more frequently in pRCC2, and that GLUT1 and MCT1 expression seem to associate with more aggressive behaviour of pRCC, indicating that metabolic markers may be useful to characterize pRCC subtypes and their behaviour. Importantly, clarification of metabolic features might improve the therapeutical management of patients harbouring pRCC**.**

No conflict of interest

B1. MAP3K6 expression and methylation profile in sporadic Gastric Cancer

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Introduction: Every year, >1 million new Gastric Cancer (GC) cases are diagnosed and around 700.000 GC-related deaths are reported. Despite all efforts, much remains to be known on the molecular events involved. Important lessons can be learned from studies of familial cases as the genes and mechanisms are frequently shared with those found in the sporadic setting. Recently, MAP3K6 has been implicated in familiar GC after the finding of several germline-mutations. Somatic hypermethylation of an intragenic CpG island overlapping a DNase-hypersensitive site predicted to harbour a promoter-associated regulatory element has been presented as a putative second-hit. The aim of the present work was to evaluate the relevance of MAP3K6 in sporadic GC. Materials and Methods: MAP3K6 expression was evaluated in RNA from a cohort of sporadic GC, as well as in corresponding adjacent normal mucosa, by qRT-PCR. Methylation profile of MAP3K6 promoter and gene-body was assessed by RRBS. Results: Overall, MAP3K6 was consistently downregulated in GC samples in comparison to corresponding adjacent mucosa. While no relevant alterations were detected in the intragenic CpG island methylation profile, promoter hypomethylation was observed in a large set of GC cases. Conclusions: MAP3K6 downregulation seems to be a common event in GC, correlating with promoter hypomethylation. These observations suggest the existence of a repressor binding site within MAP3K6 promoter, which becomes active upon demethylation. Further studies are warranted to corroborate these findings as well as to disclose the role of MAP3K6 in gastric carcinogenesis. The study was supported by: (1) FEDER funds through the Operational Programme for Competitiveness Factors—COMPETE and National Funds through the FCT—Foundation for Science and Technology, under the projects "PEst-C/SAU/LA0003/2013"; (2) NORTE-07-0162-FEDER-00018—Contributos para o reforço da capacidade do IPATIMUP enquanto actor do sistema regional de inovação" and NORTE-07-0162-FEDER-000067—Reforço e consolidação da capacidade infraestrutural do IPATIMUP para o sistema regional de inovação", both supported by Programa Operacional Regional do Norte (ON.2—O Novo Norte), through FEDER funds under the Quadro de Referência Estratégico Nacional (QREN); (3) Coimbra Genomics and BGI in the frame of the project "NGSGC—Using NGS to uncover structural and regulatory variation in gastric cancer"; (4) FCT Fellowships (SFRH/BPD/89764/2012 to PO; SFRH/BPD/86543/2012 to JC; SFRH/BPD/79499/2011 to HP) and Salary support to PF and GMA from iFCT Programmes 2014 and 2013, POPH—QREN Type 4.2, European Social Fund and Portuguese Ministry of Science and Technology (MCTES).

No conflict of interest

B2. DIES1/VISTA: A novel player in gastric cancer

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Introduction: Dies1/VISTA induces terminal differentiation of embryonic stem cells into neurons or cardiomyocytes, via the BMP-signalling pathway, but also acts as inflammation regulator and immune-response modulator. Antibody-mediated inhibition of Dies1 in a melanoma mouse model increased tumour-infiltrating T-cells and decreases tumour growth, supporting its role in immune cells from the microenvironment. The processes in which Dies1 is involved somehow mimic those associated with Epithelial-to-mesenchymal transition (EMT), namely cell de/differentiation, inflammation and cancer. Despite the common axis linking Dies1 with EMT and cancer, the expression modulation of Dies1 and the mechanisms that drive its expression in these contexts are unknown. Materials and Methods: We therefore analysed Dies1 expression, with qRT-PCR, its regulation by promoter methylation analysis and miR-125a-5p expression, and its relationship with the BMP-pathway via expression analysis of ID1/ID2/ID3 downstream targets, in a TGFβ1-induced EMT model, epithelial cancer cell lines and primary samples, aiming at disclosing a role for this molecule in epithelial carcinogenesis. Results and Discussion: We disclosed promoter methylation as the epigenetic mechanism that controls Dies1 expression in an inflammation-induced EMT model and in several epithelial cancer cell lines. We have also shown that the relationship between Dies1 expression and BMP-pathway found for the EMT model may be mimicked, or not, in different cancer types. This suggests that Dies1 may have cell-specific effectors, beyond the BMP pathway. We further demonstrated that: 1) Dies1 loss of expression is a recurrent event in GC that is caused by promoter methylation and/or miR-125a-5p overexpression; 2) ID3 is likely a downstream factor of Dies1 in GC, and;3) myofibroblasts from the GC microenvironment overexpress Dies1. Taken together, our findings identify Dies1 is a novel player in carcinogenesis, and support distinct roles for this molecule within tumour cells and in the tumour microenvironment.

No conflict of interest

B3. Uncovering the potential of THOR as a biomarker for breast cancer

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Introduction: Breast cancer (BC) is the most common cancer among women worldwide. Early detection and improved treatment have led to an increase in overall survival, however, many women are still diagnosed after tumor progression and in these cases the overall survival decreases drastically. Furthermore, since tumor relapse represents the main cause of death from this disease, the identification of biomarkers that could predict tumor behavior is a major issue in this pathology. The most cancers are able to recur by gaining limitless self-renewal, being this hallmark of cancer extremely related with telomere maintenance. Telomerase, the enzyme that maintains telomeres, is active in 90% of malignant cancers, but not in most normal tissues, making it an attractive biomarker for cancer. Telomerase activation occurs through human Telomerase Reverse Transcriptase (hTERT) expression and it has been reported that it is markedly increases in tumor invasion, which is associated with a poor BC prognosis. The mechanism of hTERT regulation is poorly understood, however, it has been reported that hypermethylation of CpG islands plays an essential role for hTERT expression in telomerase-positive BC cells. Our group recently identified a specific region in the hTERT promoter (termed THOR) which is hypermethylated and associated with telomerase activation in cancer tissue. THOR (TERT Hypermethylated Oncological Region) predicted tumor progression and patient outcome in several pediatric and adult tumors. These exciting findings led us to hypothesize that THOR is a cancer signature and may represent a diagnostic and prognostic tool, as well as a therapeutic target for BC. Materials and Methods: Using The Cancer Genome Atlas (TCGA) data we analyzed THOR status for the breast invasive carcinoma cohort (n=872 human samples). Level 3 methylation data was extracted via the UCSC Cancer Genome Browser. This

dataset includes Illumina Infinium HumanMethylation450k array beta values ranging from 0 to 1. Three informative probes illustrating differential methylation signatures in hTERT promoter were analyzed: cg17166338, cg11625005 (CG within THOR) and cg02545192. All statistical analysis was performed using GraphPad Prism5.0. Results and Discussion: Comparing the methylation levels of the three probes analyzed, the CpG site within THOR (cg11625005) was the only one that differed (p<0.0001) between malignant and non-malignant breast tissue, being this hypermethylation positively correlated with hTERT expression (r=0.163; p<0.0001) in cancer. According to the stage of disease and the presence of distant metastases, the average methylation values tend to be higher in the most advanced disease stage, however, these differences did not reach statistical significance. These preliminary findings are in agreement with previous data on THOR and evidence the potential value of THOR as a BC biomarker.

No conflict of interest

B4. The role of MLH1 constitutional methylation in lynch syndrome

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Introduction: Lynch syndrome (LS) is the most common hereditary syndrome that predisposes to colorectal cancer (CRC), being associated with 2-5% of all CRCs. It is an autosomal dominant disease caused by germline mutations in the Mismatch Repair (MMR) genes. These include MLH1, MSH2, MSH6 and PMS2, and about 90% of the mutations described in this syndrome occur in MLH1 or MSH2. There are cases described in the literature with clinical criteria for LS without germline mutation in MMR genes, which have "constitutional epimutations" or "'epimutations" in MLH1 (usually primary) or MSH2 (usually secondary). This phenomenon consists in transcriptional silencing of the promoter of these genes by epigenetic mechanisms rather than by genetic mutations that directly affect the sequence of the gene. Material and Methods in a series of 38 patients who meet clinical criteria for testing for LS, with loss of MLH1 expression in the tumor and with no germline mutation in MLH1 (35/38) or with somatic BRAF V600E mutation (3/38), we screened for constitutional methylation of the MLH1 promoter using methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) in different biological samples. Results and Discussion We found four (4/38; 10.5%) patients with constitutional methylation in the MLH1 promoter in mosaicism. RNA studies demonstrated a decreased MLH1 expression in the cases with constitutional methylation when compared with controls, and in two cases (heterozygous for a coding polymorphism) we could demonstrate that this reduction in expression was monoallelic. These results indicate that the constitutional MLH1 promoter methylation directly correlates with a reduction of gene expression. All tumors of the patients harboring constitutional methylation were microsatellite instability-high (MSI-H) and did not present the p. Val600Glu BRAF mutation. In addition, we were able to study three relatives of one of the probands (parents and sister) and constitutional methylation was not detected in any of them, suggesting that the methylation arose de novo in this proband. We conclude that a significant proportion of patients with Bethesda criteria who have loss of MLH1 protein expression and who do not have a MLH1 pathogenic germline mutation present constitutional MLH1 methylation. The inclusion of this analysis in the diagnostic strategy of LS patients increases the diagnostic yield and allows screening and/or prophylactic measures according to risk.

No conflict of interest

B5. THOR methylation as a new tool for the clinical management of colorectal cancer

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Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide with nearly 1.4 million new cases diagnosed in 2012. Despite technological advances, there is a lack of biomarkers able to identifying patients who are at risk of tumor progression or recurrence in particularly patients with disease at Stage II where therapeutic decisions are still challenging. Most cancers are able to recur by gaining limitless self-renewal. This hallmark of cancer is governed by telomere maintenance and constitutive telomerase activation through TERT expression. In 90% of human tumors, maintenance of telomeres is achieved through activation of telomerase. Our group recently identified a specific region in the hTERT promoter termed THOR (TERT Hypermethylated Oncological Region) which is hypermethylated and associated with telomerase activation in cancer tissues (Castelo-Branco P. et al, Lancet Onc, 2013). Clinically, THOR could also predict tumor progression and patient outcome in several pediatric and adult tumors. These findings led us to hypothesize that THOR is a cancer signature and may represent a new tool for diagnosis/prognosis, predicting cancer aggressiveness in CRC. Materials and Methods: Using The Cancer Genome Atlas (TCGA) data we assess THOR methylation levels and TERT expression in CRC cohort (n=434 patients). Genome-wide methylation measures were generated using the HumanMethylation450 array at cg11625005 (CG site within THOR). For evaluation of TERT expression we used the lluminaHiSeq 2000 RNA Sequencing data. A second cohort of CRC patients (n=240) was selected from the Gastroenterology Service at CHAlgarve in order to identify the predictive value of common CRC biomarkers (CEA, CA 19.9 and KRAS) in low and intermediate stages of the disease. Statistical analysis was performed using GraphPad Prism5.0. Results and Discussion: THOR was differentially methylated between normal and tumor tissues (p<0.0001), particularly in patients with Stage II of the disease and positively correlated with TERT expression (p<0.0001). Moreover, we observed an increase in THOR levels has disease progresses. Analysis of common CEA, CA 19.9 and KRAS markers for CRC in the CHAlgarve cohort revealed that significant abnormal levels of these biomarkers was only observed at stage IV of the disease. These results suggest that THOR could add prognostic/diagnostic value to patients with CRC where therapeutic decisions are yet to be consensual.

No conflict of interest

B6. Germline Variants in DNA interstrand-cross link repair genes may contribute to increased susceptibility for serrated polyposis

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Introduction: Serrated polyposis (SP) is characterized by the development of multiple colorectal serrated polyps and increased predisposition to colorectal cancer (CRC). However, the molecular basis of SP, especially in cases with family history of polyps/CRC in first degree relatives (SP-FHP/CRC), is still unknown. In a previous study, we reported that SP-FHP/CRC patients present clinical and histological differences when compared to apparently sporadic SP patients. In addition, we proposed the existence of two molecular entities amongst SP-FHP/CRC families, proximal and distal SP-FHP/CRC, according to the preferential location of lesions and somatic events involved in tumor initiation: MGMT and mismatch repair (MMR) gene defects and Wnt gene mutations, in the former; mutations in the RAS/RAF genes in the latter. This points out for the involvement of distinct tumorigenic pathways in these two forms of SP-FHP/CRC and led us to suggest that the early MGMT and MMR gene deficiency may underlie an inherited susceptibility to genotoxic stress in the proximal form. In the present study, we aimed to characterize these distinct subgroups of SP-FHP/CRC at the germline level by analyzing a panel of 94 genes associated to increased cancer risk. Materials and Methods: NGS was performed using

TruSight Cancer panel, in 10 SP-FHP/CRC patients (6 with proximal and 4 with distal SP-FHP/CRC) and in 3 sporadic SP patients previously studied. Results and Discussion: Likely pathogenic germline variants in genes coding for proteins involved in the Fanconi Anemia (FA) pathway, that act downstream of FA complex to facilitate DNA Interstrand-Cross Link repair (ICLR), were detected in 4/10 SP-FHP/CRC patients. These variants were found only in the proximal group (4/6). We found mutations in genes coding for DNA nucleotide excision repair (NER) proteins in 2/3 apparently sporadic SP patients. DNA damage caused by alkylating agents, if not repaired by MGMT and MMR, may lead to DNA double-strand breaks. The latter, together with defects in DNA-ICLR pathway, will result in elevated chromosomal/DNA breakage and genome instability, consistent with the mutation signature previously reported by us in the proximal SP-FHP/CRC group. Therefore, germline defects in DNA-ICLR genes, identified in this study, may contribute to increase serrated colorectal polyps/carcinoma risk in a subgroup of SP-FHP/CRC. Moreover, defects in NER genes may account for a subgroup of apparently sporadic SP patients.

No conflict of interest

B7. Prevalence of germline TP53 mutations in early-onset HER2-positive breast cancer

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Introduction: Breast cancer is the single most frequent event in Li-Fraumeni syndrome, accounting for more than 25% of all tumors in affected families. This syndrome is a rare inherited cancer susceptibility syndrome associated with germline mutations in the TP53 gene. Recent studies have been shown that breast cancers in women with Li-Fraumeni syndrome are commonly hormone receptor and HER2-positive, the latter being rare in BRCA1/2 mutation carriers, suggesting that HER2 amplification or over-expression in a young woman may be a useful criterion for identifying carriers of germline TP53 mutations. Materials and Methods In order to identify the contribution of germline TP53 mutations for early-onset HER2-positive breast cancer, we performed Sanger sequencing in peripheral blood samples from 78 women with HER2-positive breast cancer diagnosed until the age of 40. Results and Discussion Two heterozygous mutations were found in two patients, the c.524G>A, p.Arg175His, and the c.642T>G, p.His214Gln. The former is a pathogenic mutation, largely described in the literature as a germline mutation hotspot, and the later is a variant of unknown significance. These two mutations occur within the DNA binding domain of the TP53 gene, located at exons 5-8. Recent data show that mutations in this domain frequently interfere with the DNA binding capability or disrupt the structure of the binding surface, affecting the protein ability to regulate transcription of target genes and leading to the loss of the ability to mediate most, if not all, of TP53 functions. We conclude that, although the majority of breast cancers in women with germline TP53 mutations are HER2-positive, their frequency in early-onset HER2-positive breast cancer is relatively low. However, taking into account the potential clinical impact, women diagnosed with HER2-positive breast cancer at a young age should receive genetic counseling and genetic testing that includes TP53.

No conflict of interest

B8. Study of TERT promoter mutations in Portuguese families with Familial Non-Medullary Thyroid Carcinoma (FNMTC)

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Introduction: The genes causing Familial Non-Medullary Thyroid Carcinoma (FNMTC) that have been identified to date are only involved in a small number of families, and for the majority of the cases the molecular basis is largely unknown. Previous studies have reported shorter relative telomere length (RTL), telomerase reverse transcriptase (TERT) gene amplification and increased expression, in leukocytes from FNMTC patients, when compared with sporadic NMTC. Recently, somatic mutations in the promoter region of TERT, which increase promoter activity, were reported in sporadic thyroid tumours. In some studies, TERT promoter mutations were found to correlate with shorter telomeres, and more aggressive features when coexisting with BRAF mutations. The aim of the present study was to investigate the role of TERT promoter mutations in the aetiology and progression of FNMTC. Materials and Methods The promoter region of TERT (encompassing 330 bp upstream of the ATG and 37 bp of exon 2 of the gene) was sequenced in leukocyte DNA of the probands from 60 FNMTC families. In addition, TERT promoter (upstream from the ATG start site) and BRAF V600E mutations were also assessed in 43 familial thyroid tumours [25 papillary thyroid carcinomas (PTC) of the classic subtype, 9 follicular variants of PTC, 3 mixed PTC, 3 oncocytic variants of follicular thyroid carcinomas, 2 tall-cell PTC, and 1 classic PTC with Hürthle cells] from 32 probands and 8 affected family members. Results and Discussion No potentially pathogenic germline variants were identified in TERT in the 60 probands from the FNMTC families. A common promoter polymorphism rs2853669 (T>C), which has been suggested to act as a modifier of TERT mutations effect, was detected in 45% of the probands. In the 43 thyroid tumours, we identified 4 samples (9.3%) with hotspot somatic TERT promoter mutations in the positions -124bp (C>T; 3 samples) and -146bp (C>T; 1 sample), which are expected to confer enhanced TERT promoter activity, putatively by generating a consensus binding site for E-twenty-six/ternary complex factors (Ets/TCF). TERT positive samples were also positive for BRAF V600E. These preliminary results suggest that TERT promoter mutations are not frequently involved in FNMTC aetiology, and that they are rather involved in tumour progression, and more often in concomitance with BRAF mutations.

No conflict of interest

B9. Germline variants in HR-mediated DNA damage repair genes may contribute to increased crc susceptibility in a subgroup of FCCTX

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Introduction: Familial colorectal cancer type X (FCCTX) families are clinically defined by the Amsterdam criteria, the absence of germline mutations in mismatch repair (MMR) genes and the presence of microsatellite stable tumors. We have previously reported the presence of two distinct molecular entities amongst tumors from 15 FCCTX families from our registry: one (n=10) whose tumors presented frequent loss of heterozygosity in tumor suppressor genes (TSG+) and another (n=5) with tumors lacking this molecular feature. Amongst TSG+ tumors, we found a subgroup (n=7) with a prevalence of APC/KRAS somatic mutations and MMR/MGMT methylation, and a second, where these features were almost absent. In the present study we aimed to characterize these distinct subgroups at the germline level by the analysis of a multigene panel of 94 genes associated to increased cancer risk. Materials and methods: NGS was performed using the TruSight Cancer panel in the 15 index patients previously studied. Large deletions were evaluated for all genes associated with hereditary colorectal cancer syndromes by MLPA. Likely pathogenic variants were confirmed by Sanger sequencing, and segregation analysis in the respective family was performed, whenever DNA samples were available. Results and discussion: In 7/15 families, all TSG+, we found one or more likely pathogenic variants in genes encoding proteins involved in double strand breaks (DSB)-associated DNA repair pathways, secondary to DNA damage response to genotoxic stress, particularly in homologous recombination (HR)-mediated DNA damage repair. Six of the seven families belong to the subgroup whose tumors presented frequent KRAS somatic mutation and/or MGMT/MMR gene methylation. In two of these families we have also detected a likely pathogenic missense mutation in BMPR1A gene and a deletion of SMAD4 exons 5-8, respectively. The cytotoxic effects of alkylating agents, if not repaired by MGMT and MMR system, will eventually lead to DNA DSB. The latter, together with

defects in HR-DNA repair pathways, will result in elevated chromosomal/DNA breakage and genome instability, which are consistent with the mutation signature previously reported by us in the FCCTX TSG+ subgroup. Therefore, germline defects in HR-DNA repair genes, identified in the present study, may contribute to increase colorectal adenoma/carcinoma risk in a subgroup of FCCTX families with TSG+ tumors, carrying frequent KRAS mutations and/or MGMT/MMR gene methylation.

No conflict of interest

B10. Can Polymorphisms in oxidative stress related genes be prognostic factors in CML?

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Introduction: Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell damage, apoptosis and ineffective hematopoiesis. Chronic myeloid leukemia (CML) is a clonal neoplastic disease associated with the reciprocal translocation t(9;22), encoding the BCR-ABL1 oncogene. BCR-ABL protein induces, among other mechanisms, production of reactive oxygen species (ROS) by activation of the PI3K pathway, increase glucose metabolism and mitochondrial dysfunction. The antioxidant enzymes superoxide dismutases (SOD) and catalase (CAT), as well as DNA repair enzymes, such as OGG1, are important cell defense components against OS. Polymorphisms in genes that codify these enzymes may contribute to differences in susceptibility of individuals to oxidative damage, since it can lead to reduced protection against OS, influencing CML development and therapeutic response. In the present study we investigate the influence of polymorphisms in genes related with oxidative stress (CAT, GPX1, MPO, SOD1, and SOD2) and DNA repair (OGG1, NEIL1, and XRCC1), and the transcription factor NFE2L2, as a prognostic risk marker in CML patients [namely in overall survival and tyrosine kinase inhibitors (TKIs) response]. Moreover, we also analyzed it participation in the development of mutations in BCR-ABL1 gene. Materials and Methods: This study enrolled 75 patients diagnosed with CML. The genetic polymorphisms of CAT (rs1001179), GPX1 (rs1050450), MPO (rs2333227), SOD1 (rs2070424), SOD2 (rs4880), OGG1 (rs1052133), NEIL1 (rs5745920), XRCC1 (rs1799782), and NFE2L2 (rs13001694), were assessed by RFLP-PCR and Tetra-primer-ARMS-PCR. The statistical analysis was carried out by variance analysis, $\chi 2$ test and Fisher exact test ($p<0.05$). Results and Discussion: Our results show that SOD2 genotype influence mutation status of BCR-ABL1 (CC genotype: odds ratio 9.25x, I.C.95% 1.24-18.82; p=0,007). On the other hand, patients with MPO GG and AG genotypes have a high rate of sub-optimal response to TKIs (odds ratio 4.92x, I.C.95% 1.24-9.10; p=0,043). Moreover, the overall survival of CML patients can be influenced by NEIL1 [CML patients with CT genotype had lower survival (166 ± 5 months) than patients with CC and TT genotypes (204 ± 6 months; p=0.041)] and NFE2L2 [CML patients with TT genotype had lower survival (88 ± 7 months) than patients with CT and TT genotypes (216 ± 8 months; p=0.003)]. These results suggest that genetic polymorphisms in oxidative stress and DNA repair related genes influence the prognosis of CML patients, the TKI response and the development of BCR-ABL1 mutations, which could constitute new prognostic and therapeutic biomarkers. This work was supported by CIMAGO (Project 22/09) and R. Alves is supported by the FCT fellowship SFRH/BD/51994/2012.

No conflict of interest

B11. Are Dietary Folate Intake, MTHFR polymorphisms and Breast Cancer Risk associated? A meta-analysis

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Introduction: Breast cancer incidence has been increasing by more than one million new cases every year and is a primary cause of cancer death among women worldwide. Folate plays an important role in DNA methylation, synthesis, and repair; intake has been associated with breast cancer. Methylenetetrahydrofolate reductase (MTHFR) gene is polymorphic at nucleotides 677 (CT) and 1298 (AC), resulting in allozymes with decreased activity. Several studies have pointed to association between the MTHFR C677T polymorphism and breast cancer risk. Objectives: The present study aims to contribute to the elucidation of the impact of any C677T breast cancer association through a meta-analysis study of published case control studies. Methods: Pubmed, Google Scholar, Elsevier and Cochrane trials databases were searched for case control studies of associations between MTHFR C677T polymorphism and breast cancer risk. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association. Results and Discussion: MTHFR C677T polymorphisms may modify the association between dietary folate intake and breast cancer risk. Homozygous women for the MTHFR 677T polymorphism with deficient dietary folate intake may have a significantly increase of breast cancer risk.

No conflict of interest

B12. Controversial Use of Antidepressants and Breast Cancer Development – a meta-analysis

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Introduction: Breast cancer is the most prevalent cancer in women worldwide and there is considerable research proving that depression is an aetiological factor in the development of cancer and a risk factor concerning mortality of breast cancer. Studies in rats also demonstrated a possible development of breast cancer due to antidepressant use. Moreover, even though studies in humans are trifling, the few studies examining the relationship between antidepressant use and breast cancer risk have produced conflicting results. Objectives: The goal of the present study was to analyze associations between use of antidepressant medication and risk of breast cancer. A second goal was to test whether these associations would vary depending on study characteristics. Materials and Methods: We review evidence for the study goal based upon prospective longitudinal studies. We searched in MEDLINE, EMBASE and Cochrane trials registers from December 2014 and January 2015 for studies studying association of antidepressants and breast cancer. Bibliographies of elected articles were examined for further references. Research took into account a predetermined list of Medical Subject Headings (MeSH) and free text terms where appropriate. We combined the terms "antidepressive agents", "depression ", ''antidepressant use" and "breast cancer risk". Results and Discussion: Meta-analysis was used for integrating the results of 12 eligible trials of anti-depressive treatments. There is some evidence that there exist association between antidepressant use and breast cancer. Further confirmatory trials and research are required before the body of scientific evidence can be conclusive. Conclusion: There is no evidence that antidepressant use is (not) associated with breast cancer development.

No conflict of interest

B13. Microsatellite instability-low and loss of heteroziigozity in 2P may underlie increased susceptibility for familial rectal cancer

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Introduction: Revised Bethesda guidelines (BG) identifies colorectal cancer (CRC) patients suspected of Lynch syndrome (LS), that do not fulfill Amsterdam criteria, to be tested for microsatellite instability (MSI) in the respective tumors, an hallmark of LS. Patients fulfilling BG whose tumors present MSI-high (MSI-H) should undergo germline mutation analysis for DNA mismatch-repair (MMR) genes. MMR gene mutations define LS and are found in approximately 40% of patients with MSI-H tumors. However, the molecular basis underlying increased CRC susceptibility in the remaining cases fulfilling BG is still unknown. In the present study, we aimed to characterize retrospectively patients with CRC fulfilling BG but without germline MMR gene mutations, for MSI status, namely regarding dinucleotide (DNR) or mononucleotide repeat (MNR) sequences, and for specific BG characterization, tumor stage and location in the colon and rectum. Materials and methods: We selected 225 patients with CRC fulfilling the BG from our Familial Cancer Registry, having germline MMR gene mutations excluded: 121 microsatellite stable (MSS) and 104 MSI tumors- 63 MSI-H and 41 MSI-low (MSI-L). MSI analysis have been performed by analysing the Bethesda microsatellite markers (3 DNR – D2S123, D5S346 and D17S250 – and 2 MNR – BAT25, BAT26), using GeneScan. Loss of heterozygosity (LOH) at DNR was also evaluated. Correlation with clinical features was performed using Stata 12. Results and discussion: Of the 41 MSI-L tumors, only two were MSI at MNR, and in the MSI-H group, 3 presented MSI only at MNR, while 5 showed MSI only at DNR. Interestingly, MSI-L tumors were more associated to BG #5 (family history of CRC in 3 family members irrespective of age at diagnosis) (15/34, 44%), than MSS and MSI-H tumors that were associated to BG #1-4 (61/94, 65% and 48/55, 87%, respectively), p=0,002. MSI-L was observed mainly in DNR. Amongst MSI tumors, MSI only at DNR was associated to rectal tumors (15/21, 71%), whereas MSI at both MNR and DNR was associated to proximal colon (28/39, 71%), p=0,003. Both MSI at DNR and D2S123 LOH correlated with BC #5 (15/22, 68%, p=0.005 and 16/25, 64%, p=0.045, respectively). D2S123 LOH also correlated with earlier stage tumors (33/69, 48% of stage I-III tumors and 1/11, 9% of stage IV tumors, p=0.035). Our results suggest that MSI at DNR and D2S123 LOH may underlie a specific familial susceptibility for rectal cancer with a lower tumor burden at diagnosis.

No conflict of interest

B14. :Methylation levels of two microRNAs accurately identifies urothelial cell carcinomas

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Urothelial carcinomas, including those from bladder (BUC) and upper urinary tract (UTUC), are the most common type of cancer from the urinary system worldwide. The lack of accurate early detection tools for these tumours, especially those of the upper tract, entails delay in diagnosis, precluding more efficient and timely treatment. In a previous study of ours, two microRNAS (miR 129-2 and miR 663a) were found to be differentially methylated in BUC compared to other genitourinary tract malignancies. Herein, we analysed the methylation levels of those two microRNAs, using real-time quantitative methylation-specific polymerase chain reaction (qMSP-PCR), in a series of tissue samples from BUC and UTUC, as well as normal bladder and uretheral mucosas (which served as controls). To determine the performance of both microRNAs as diagnostic or prognostic biomarkers, receiver operator characteristics (ROC) curve and survival analyses were performed, respectively. In line with our previous findings, we found that methylation levels of both miR 129-2 and miR

663a were significantly higher in BUC and UTUC compared to normal urothelial mucosa (P <0.001). This panel, accurately identified urothelial carcinoma with 82% sensitivity and 96% specificity, corresponding to an area under the curve (AUC) of 0.943 (P <0.001). These results support a common carcinogenic pathway for the urothelium, whether from the bladder or upper urinary tract, offering the possibility of accurate detection in a single molecular test. The performance of this miR promoter methylation panel compares well with other promising epigenetic based biomarkers and may constitute the basis for a clinical assay. However, its usefulness as screening tool requires the validation of these results in urine samples, allowing for non-invasive testing of urothelial cancer suspects.

No conflict of interest

B15. Could myofibroblasts fulfil a pivotal role in lung cancer progression from a fibrotic microenvironment?

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Could myofibroblasts fulfil a pivotal role in lung cancer progression from a fibrotic microenvironment? Mariangela Natale1,2, 3Sylvia Vong, 1Komal Vadnagara, 4Lina Carvalho, 2João Nuno Moreira, 1Valerie LeBleu and 1,2Raghu Kalluri 1MD Anderson Cancer Center; Houston, Texas, USA 2Center for Neuroscience and Cell Biology, CNC, Portugal; 3Harvard Medical School, Boston, USA; 4Faculty of Medicine, University of Coimbra, Portugal Introduction: Fibroblasts are the non-vascular, non-epithelial and non-inflammatory cells of the connective tissue, and its principal cellular component. Almost all connective tissues seem to be under some sort of mechanical tension even at rest. An example of tension development in connective tissue is represented by wound contraction, a process that occurs during healing of an open wound. Tissue tension is an essential regulator of tissue function, such as in lung alveoli, kidney capsule, granulation tissue contraction and uterine involution. Modified fibroblasts were first observed in granulation tissue of healing wounds, suggesting their involvement in the production of the contractile force associated with that process. Over the last few decades idiopathic pulmonary fibrosis (IPF) has been increasingly recognized as a major unmet medical need in respiratory medicine. This is due to the fact that IPF incidence is increasing worldwide, with rates similar to those of many forms of cancers. Idiopathic pulmonary fibrosis (IPF) is characterized by progressive fibrosis, with excessive matrix deposition leading to destruction of lung architecture and, ultimately, fatal impairment of lung function. Cancer, defined by some authors as a "wound that does not heal" has an often unknown etiology, risk factors similar to IPF, and the presence of a specific genetic background considered important for the occurrence of the disease. Carcinoma cells, in the lung and other organs, are closely associated with the extracellular matrix (ECM), mesenchymal cells such as fibroblasts, infiltration of immune cells and vasculature. In some cases this environment is essential to tumour initiation or growth, whereas in other cases it can prevent tumorigenesis or even promote tumour clearance. Current understanding on the cells of origin for lung cancer is mostly derived from experimental data using genetically engineered mouse models (GEMMs) that would not be possible using patient samples or cancer cell lines. The staining pattern of a tumour, from patient samples or cancer cell lines, is merely a snapshot of the gene expression of the tumour cells at that time point and might not match the initiating cell type. Non-small cell lung cancer (NSCLC) is recognized to be the most frequent type of lung cancer ($\approx$ 80%) with a major prevalence of lung adenocarcinoma subtype. Although up to 60% of lung adenocarcinomas have known oncogenic mutations, mainly in receptors or protein kinases involved in signaling pathways such as RAS-RAF-MEK-ERK or MAPK, PI3K-mTOR or JAK-STAT pathways, 40% of them are characterized by unknown mutations. It is notable that changes in the cells of origin were evident upon either alteration of the genotype for tumour initiation was altered or presence of injury or inflammation during tumour initiation. Considering that the cell of origin in lung cancer is still unknown, this study aims at getting additional insights on a new type of cell population that could potentially fulfill this role. Material and Methods: Several patient-derived lung cancer biopsies were used to establish primary cell culture lines to further perform epigenetic analyses. In parallel to gain a better understanding on the functions of microenvironment a genetic mouse model (GEMM) was engineered. A complete characterization of this model was conducted, starting from phenotype analysis (immunohistochemical staining) to cellular and molecular analysis (microarray and exome sequencing analysis; PCR; qPCR; western blot; immunofluorescent staining; FACS; immunotyping analysis). Different pharmacological treatments were conducted in vitro and in vivo based

on achieved results. Results and discussion: Epigenetic differences between normal and diseased primary cell lines established from human biopsies were observed. Interestingly the characterization of our GEMM seemed to well recapitulate all the different stages of lung cancer progression underling different pathways possibly involved in tumor progression. Altogether these data point out myofibroblast as a new cell of origin of lung cancer progression starting from a fibrotic microenvironment.

No conflict of interest

C1. RAC1b overexpression in papillary thyroid carcinomas: an activator of NF-κB pathway that contributes to thyroid tumorigenesis?

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Introduction: RAC1b is a hyperactive splicing variant of the small GTPase RAC1. Tumor-associated RAC1b overexpression has been recently highlighted as one of the most promising targets for therapeutic intervention in colon, breast, lung and pancreatic cancer. We have recently shown that RAC1b is overexpressed in a subset of papillary thyroid carcinomas (PTCs) associated with unfavorable outcome. Materials and methods: The PTC-derived K1cell line, negative for RAC1b expression, was used as in vitro model and the effects of RAC1b were assessed upon transient transfection. Activity of NF-κB and cyclin D1 signaling was assessed by luciferase-reporter assays and NF-κB nuclear localization was evaluated by immunofluorescence microscopy. The effects of RAC1b on cell cycle progression and apoptosis were evaluated by flow-cytometry. Immunohistochemical analysis of formalin-fixed paraffin-embedded tissues was also used to estimate NF-κB/p65 expression and nuclear localization in PTC samples. Results: Ectopic expression of RAC1b was able to induce a significant increase on NF-κB and cyclin D1 reporter activity. A clear nuclear localization of NF-κB/p65 was found in cells transfected with RAC1b, confirming NF-κB canonical pathway activation. Consistently, we were able to observe a RAC1b-mediated decrease in IκBα (NF-κB inhibitor) protein levels. Also, a nuclear localization of NF-κB/p65 was found to be prevalent in RAC1b-overexpressing tumors, compared to those that are negative for RAC1b expression. Finally, RAC1b overexpression was shown to protect thyroid cells against apoptosis and stimulate G1/S progression, through a process involving the NF-κB pathway. Discussion: These findings suggest that RAC1b plays an important role in papillary thyroid carcinomas and point out NF-κB activation as one of the molecular mechanisms associated with RAC1b overexpression and downstream signaling in thyroid tumorigenesis.

No conflict of interest

C2. Notch Signaling Dynamics in the Adult Healthy Prostate and in Prostatic Tumor Development

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Introduction: The Notch signaling pathway has been implicated in prostate development, maintenance and tumorigenesis by its key role in cell-fate determination, differentiation and proliferation. Therefore, we proposed to analyze Notch family member's transcription and expression, including ligands (Dll1, 3, 4 and Jagged1 and 2), receptors (Notch1–4) and effectors (Hes1, 2, 5 and Hey1, 2, L), in both normal and tumor bearing mouse prostates to better understand the dynamics of Notch signaling in prostate tumorigenesis.

Materials and Methods- Wild type mice and transgenic adenocarcinoma of the mouse prostate model (TRAMP) mice were sacrificed at 18, 24 or 30 weeks of age and the prostates collected and processed for either whole prostate or prostate cell specific populations mRNA analysis and for protein expression analysis by immunohistochemistry and immunofluorescence. Results and Discussion-. We observed that Dll1 and Dll4 are expressed in the luminal compartment of the mouse healthy prostate, whereas Jagged2 expression is restricted to the basal and stromal compartment. Additionally, Notch2 and Notch4 are normally expressed in the prostate luminal compartment while Notch2 and Notch3 are also expressed in the stromal layer of the healthy prostate. As prostate tumor development takes place, there is up-regulation of Notch components. Particularly, the prostate tumor lesions have increased expression of Jagged1 and 2, of Notch3 and of Hey1. We have also detected the presence of activated Notch3 in prostatic tumors that co-express Jagged1 and ultimately the Hey1 effector. Taken together our results point out the Notch axis Jagged1-2/Notch3/Hey1 to be important for prostate tumor development and worthy of additional functional studies and validation in human clinical disease.

No conflict of interest

C3. Characterization of a Mex3a knockout mouse model: linking intestinal stem cell homeostasis and cancer

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Introduction: Stem cells play a fundamental role in replacing multiple cell types throughout life in nearly all adult tissues but their exclusive and powerful ability for self-renewal is replicated in cancer. We have previously shown that the human RNA-binding protein MEX3A regulates intestinal differentiation, polarization and stemness associated with gastrointestinal carcinogenesis. Our aims are to determine if MEX3A protein constitutes a novel intestinal stem cell (ISC) marker with functional relevance for intestinal development and to assess for the first time the impact of MEX3A loss in intestinal regeneration upon injury and in response to carcinogenic stimuli. MATERIAL AND METHODS: To track MEX3A major biological functions, we are characterizing the very first knockout mouse model harbouring a LacZ-tagged Mex3a targeted deletion. In parallel, we are establishing ex vivo cultures of murine intestinal organoids by isolating intestinal crypts from mice with different genotypes. This system allows long term maintenance of crypt-villus axis functional equivalents and their interrogation concerning alterations in relevant signalling pathways. RESULTS AND DISCUSSION: Homozygous mutants are smaller than their heterozygous and wild-type siblings since birth and show post-natal lethality (ranging from 15 to 25 days, N=8). Histological analysis of the Mex3a-/- mice intestine revealed an overall reduction in crypt size according to age and individual. Upon examination of the intestinal epithelium differentiation pattern, the most striking features were strong reduction in the number of Paneth cells as assessed by lysozyme staining, and an increase in Goblet cell number and size assessed by Alcian Blue-PAS staining. These alterations in secretory cell types were accompanied by an overall reduction in the number of Ki67+ proliferating cells and in SOX9 expression, a transcriptional target of Wnt signalling. The results so far suggest two key phenotypes: on one hand absorption deficiency due to an imbalance in intestinal cell lineages and on the other hand a severely impaired cell renewal probably due to loss of stem cell potential. This is further reinforced by our preliminary data with intestinal cell sorting, showing that Mex3a expression is restricted to stem and Paneth cells, which constitute the ISC niche. We are now assessing the presence of stem cells by performing RNA in situ hybridization for Olfm4, a standard marker for visualization of ISCs. The results obtained so far lead us to hypothesize that MEX3A is a key regulator in the transition from progenitor to differentiated cells in the intestinal epithelium, with putative implications for carcinogenesis.

No conflict of interest

C4. Dissecting the role of TRIB2 in malignant melanoma using CRISPR technology

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Introduction: Melanoma is the deadliest form of skin cancer and its incidence increased significantly in the last three decades [1]. Despite the knowledge of key pathways involved in this disease, advanced melanoma is still refractory to a variety of therapies. TRIB2, a gene encoding one of the three proteins of tribbles family, is highly expressed in melanoma [2]. Recently, TRIB2 was proved viable as a biomarker for diagnosis and progression of metastatic melanoma [3]. Furthermore, data from our lab shows that TRIB2 has been implicated in resistance mechanisms to PI3K inhibitors trough promotion of AKT activity (Unpublished data). Still, the mechanisms by which TRIB2 mediates melanoma progression and chemoresistance are largely unknown. Interestingly, the remaining proteins of this family, TRIB1 and TRIB3, show high similarity to TRIB2 and have been implicated in other malignancies such as leukemia and colorectal cancer [4]. In order to investigate the oncogenic role of TRIB2 in melanoma we took advantage of the newly gene-engineering tool CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) to generate TRIB1, TRIB2 and TRIB3 knock-outs. Simultaneously, we generated a FLAG-TRIB2 knock-in cell line by adding 3xFLAG upstream of TRIB2. The development of these tools will be critical to study the role of TRIB2 in melanoma development and resistance to anti-cancer therapy. Material and Methods: We analysed TRIB2 expression levels in a large panel of melanoma cell lines by RT-PCR. Amongst the cell lines that express high level of TRIB2, we selected A375 to validate CRISPR-Cas9 knock-out and FLAG knock-in constructs. We designed two CRISPR/Cas9 gRNAs for each of the TRIBBLES family gene (TRIB1, TRIB2 and TRIB3). Oligo annealing and insertion into pX459-puromycin resistance plasmid was done as described previously [5], [6]. We performed colony PCR to select positive clones that were further validated by sequencing. We are currently selecting A375 single cell clones for consequent studies. Results and discussion: RT-PCR analysis showed high TRIB2 expression levels in the majority of the melanoma cell lines. We have successfully cloned all CRISPR plasmids. Preliminary results assessed by RT-PCR showed that all three TRIBBLES genes were efficaciously knock-out. The FLAG knock-in melanoma cell line will be used to identify new TRIB2 partners. With this strategy we aim to establish powerful tools that will enable us to dissect the role of TRIB2 and related family members in the context of malignant melanoma. Understanding in which signalling pathways TRIB2 is involved and how it relates to the acquired resistance to several anti-cancer agents could help us to develop more efficient approaches to treat melanoma. References: [1] American Cancer Society, "Cancer Facts & Figures 2014," Cancer Facts Fig., pp. 1–72, 2014. [2] F. Zanella, O. Renner, B. García, S. Callejas, a Dopazo, S. Peregrina, a Carnero, and W. Link, "Human TRIB2 is a repressor of FOXO that contributes to the malignant phenotype of melanoma cells", Oncogene, 2010. [3] R. Hill, R. K. R. Kalathur, L. Colaço, R. Brandão, S. Ugurel, M. Futschik, and W. Link, "TRIB2 as a biomarker for diagnosis and progression of melanoma", Carcinogenesis, 2015. [4] T. Yokoyama and T. Nakamura, "Tribbles in disease: Signaling pathways important for cellular function and neoplastic transformation", Cancer Science, 2011. [5] L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, and F. Zhang, "Multiplex Genome Engineering Using CRISPR/Cas Systems", Science, 2013. [6] F. A. Ran, P. D. Hsu, J. Wright, V. Agarwala, D. A. Scott, and F. Zhang, "Genome engineering using the CRISPR-Cas9 system.," Nature. Protocols, 2013.

No conflict of interest

C5. Do IL-7Rα gain-of-function mutants signal from intracellular compartments? Implications for acute lymphoblastic leukemia

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Introduction: The cytokine interleukin-7 (IL-7) is mandatory for normal T -cell development. However, IL -7 and its receptor (IL-7R) also partake in leukemia development. Around 10% of T-cell acute lymphoblastic leukemia (T-ALL) and a small fraction of B-cell ALL patients display IL7R gain-of-function mutations, leading to disulfide bond-dependent homodimerization of mutant receptors and ligand-independent constitutive activation of

downstream signaling. Here, we started exploring whether mutant IL-7R may trigger downstream signaling without necessarily reaching the cell surface. Materials and Methods: IL-7R-mediated signaling was assessed using Ba/F3 cells expressing either the wild-type (wt) or mutant IL-7R. Two highly conserved endoplasmic reticulum (ER) retention motifs (KDEL and KKXX) were combined to retain the IL-7R within the ER. Strategies based on proteases (trypsin and pronase E) and drugs (brefeldin A) were employed to abrogate IL-7R surface expression. The efficacy of intracellular IL-7R retention was determined by confocal microscopy and flow cytometry and its impact on IL-7R-mediated signaling measured by immunoblot determination of the phosphorylation levels of IL-7R downstream targets. Cells were isolated by FACS. Results and Discussion: ER retention motifs were not sufficient to sequester the IL-7R within the ER. However, pronase E and brefeldin A efficiently abrogated IL-7R surface expression. Notably, the relative impact of IL-7R surface depletion on IL-7R-mediated signaling was lower in cells expressing mutant IL-7R as compared to those displaying the wt receptor. In addition, by isolating a sub-population of BaF3 cells that expressed the IL-7R only in the cytoplasm, we observed that IL-7R wt-expressing cells did not respond to IL-7 stimulation and eventually died. In contrast, mutant IL-7R was able to sustain signaling and promote cell viability, growth and proliferation by itself, without the requirement to be expressed at cell surface. Altogether, our results suggest that mutant IL-7R may be able to signal, at least to some extent, before reaching the plasma membrane. Therapeutic strategies aiming at eliminating IL-7R-positive leukemias may have to take these findings into account.

No conflict of interest

C6. A novel pathway-oriented ChIP-Seq approach to study genomic reprogramming in CRC

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Introduction: Transformation of a normal cell into a cancer cell implicates a vast reprogramming of the cell's gene expression pattern, a fact that has been extensively explored by the technological breakthroughs in the areas of genomics and transcriptomics to find "gene expression signatures". Despite the development of some targeted therapies, most biomarkers are still controversial and very few have proven to be useful for clinical practice. The underlying problem relates to high heterogeneity present in biological systems and to technical difficulties in quantitatively assessing molecular alterations. Materials and Methods: We used a modified ChIP-Seq approach to address this issue, focussing the genomic analysis in one particular aspect of the cell's oncogenic reprogramming. Previously, we had shown that Rac1/PAK1 pathway, which is altered in up to 60% of solid tumours, selectively modulates the synchronized activities of the transcription factors BCL6 and STAT5 in colorectal cancer cell lines. This selective gene modulation was explored to identify Rac1/PAK1/BCL6/STAT5-specific gene targets, within the entire genome, using biological rather than a statistical filter to parameterize the ChIP-Seq analysis pipeline. Results and Discussion: The method worked well, functioning as a biological "sieve" that minimized the background "noise" and facilitated the identification of pathway-oriented candidate genes. Preliminary data analysis allowed already the unveiling of unsuspected functions of Rac1 signalling in nucleic acid metabolism.
No conflict of interest

C7. Ibuprofen Inhibits Overexpression of Tumor-Related Rac1b through SRSF1

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Introduction: The serrated pathway to colorectal tumor formation involves oncogenic mutations in the BRAF gene, which are sufficient for initiation of hyperplastic growth but not for tumor progression. A previous

analysis of colorectal tumors revealed that overexpression of splice variant Rac1b occurs in around 80% of tumors with mutant BRAF and both events proved to cooperate in tumor cell survival. Patients with inflamed human colonic mucosa also have increased expression of Rac1b as well as mice with experimentally induced colitis. The increase of Rac1b in the mouse model was specifically prevented by the nonsteroidal anti-inflammatory drug ibuprofen. Materials and Methods HT29 colorectal cell line was used as model to test several signaling pathways after 48h of treatment with ibuprofen. For this we analyzed the proteins of interest by Western Blot and the transcript levels by RT-PCR. Results and Discussion Mechanistic studies in cultured HT29 colorectal tumor cells revealed that ibuprofen inhibited Rac1b expression in a cyclooxygenase inhibition–independent manner and targets directly the alternative splicing event. Here, we provide evidence that ibuprofen leads to a decrease in expression of SRSF1, a splicing factor that we previously identified to promote Rac1b alternative splicing. Together, our results suggest that stromal cues, namely, inflammation, can trigger changes in Rac1b expression in the colon and identify ibuprofen as a highly specific and efficient inhibitor of Rac1b overexpression in colorectal tumors. Our data identify an additional cyclooxygenase–independent action of ibuprofen and suggest it may be beneficial in the treatment of patients with the subtype of BRAF-mutated serrated colorectal tumors.

No conflict of interest

C8. Targeted inhibition of PI3K p110α as an alternative therapeutic strategy for colorectal cancer patients with KRAS mutations

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer mortality worldwide. It is often associated with activating mutations in KRAS, BRAF and PIK3CA leading to the deregulation of major signaling pathways as the RAS-RAF-MAPK and PI3K-PTEN-AKT. However, despite intensive research, information is scarce regarding the molecular mechanisms underlying the survival of mutant CRC cells that are mostly resistant to the available therapies. At present, the alternative therapeutic option for patients with metastatic CRC (mCRC), in addition to the conventional therapies, involves the use of EGFR antibodies but only a small percentage of patients benefit from such therapies. Moreover, CRC patients harbouring somatic KRAS mutations are excluded from EGFR targeted therapies. Thus, it is urgent to unravel novel strategies for CRC therapy, in particular for KRAS mutant CRC patients. MATERIALS AND METHODS To elucidate the potential benefit of targeting the PI3K signaling pathway, the cellular effects of PI3K p110α specific inhibition by silencing RNA (siRNA) were assessed in human CRC cells. Moreover, the inhibition of MEK1/2 was also evaluated. Specifically, proliferation/viability, cell cycle and apoptosis were monitored upon PIK3CA and/or MEK1/2 silencing in human CRC cell lines harboring hotspot mutations in KRAS and KRAS/PIK3CA. Furthermore, the effects of BYL719, a specific PI3K p110α inhibitor, were also investigated in vitro and in vivo. RESULTS AND DISCUSSION The results demonstrate a pivotal role for PI3K in CRC cells with mutations in KRAS and KRAS/PIK3CA. More specifically, targeted depletion of PI3K p110α by siRNA reduced viability in CRC cells and induced apoptosis and cell cycle arrest in KRAS/PIK3CA and KRAS mutant CRC cells, respectively. In addition, and further supporting the observed cellular effects, silencing of PIK3CA lead to alterations in the expression levels of proteins implicated in apoptosis and cell cycle as XIAP and pBad in KRAS/PIK3CA mutant cells and cyclin D1 in KRAS mutant cells. Moreover, a specific PI3K p110α inhibitor, BYL719, was able to mimic the in vitro siRNA effects on cellular viability, apoptosis and cell cycle arrest in KRAS/PIK3CA and KRAS mutant CRC cells. In addition, BYL719 significantly reduced tumor size in mice xenografted with KRAS/PIK3CA mutant CRC cells. Overall, this study demonstrates that specific inhibition of PI3K p110α could provide an alternative therapeutic approach for CRC patients, particularity those with KRAS and KRAS/PIK3CA mutations.

No conflict of interest

C9. The role of WNT and Hedgehog signaling pathways as novel prognostic markers and therapeutic targets in pedriatic B-ALL

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Introduction: Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can arise from the aberrant activation of embryonic signaling pathways, such as Wingless (WNT) and Hedgehog (Hh) pathways. Therefore, these pathways may constitute a new therapeutic target for ALL treatment. Our goals were to establish the activation patterns of Wnt and Hh signaling pathways in pediatric B-ALL in order to identify novel prognostic markers and new targets for therapeutic strategies. Furthermore, we also want to evaluate the therapeutic potential of IWR-1 and GDC-0449, inhibitors of WNT and Hh signaling pathways, respectively, in an in vitro model of ALL. Materials and Methods: We studied 9 bone marrow (BM) samples from pediatric patients with B-ALL (average age 10,2y, 4F:5M) and 3 BM of controls (average age 9y, 3F). A Wnt and a Hh gene expression arrays (84 genes each) were performed. KOPN-8 cell line (B cell ALL model) was treated with IWR-1 and GDC-0449 inhibitors in several concentrations. Cell viability and density were evaluated by trypan blue. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. FC was also used to analyze BAX and BCL-2 expression, cell cycle (PI/RNase assay) and mitochondrial membrane potential (JC1 dye). Results and Discussion: We observed that B-ALL patients showed in Wnt signaling pathway, downregulation of some WNT (WNT10A) and FZD family (FZD5) genes, Wnt negative regulators (KREMEN1) and cell cycle target genes (CCND1), in comparison with controls. In Hh signaling pathway, these patients also presented a downregulation of some ligands (SHH), receptors (PTCHD1), regulators (GLI2), and target genes (VEGFA), as well as upregulation of others (DHH, PTCHD2, CSNK1E, BCL2), in comparison with controls. Our results showed that IWR-1 and GDC-0449 reduces viability and proliferation of KOPN-8 cells in a time and dose dependent manner, with an IC50 (48h) of 50 μM for IWR-1 and 75 μM for GDC-0449. Both inhibitors induced cell death by apoptosis, with increased BAX/BCL-2 ratio and mitochondrial membrane depolarization. Moreover, both inhibitors induce cell cycle arrest in G0/G1 phase. Our results suggest that B-ALL pediatric patients have tendentiously a downregulation of genes involved in Wnt signaling pathway and abnormal expression of genes related with Hh signaling pathway. The present results support that these pathways may provide novel prognostic markers and potential new therapeutic targets, and that IWR-1 and GDC-0449 could be new targeted therapeutic approaches in B-ALL.
No conflict of interest

C10. The role of annexin A2 in cancer cells during hypoxia

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Introduction: Solid tumors are often subjected to hypoxia (oxygen deprivation) during their progression, due to increased distance from the vasculature and deficient angiogenesis. Several studies revealed that hypoxic cancer cells typically exhibit increased levels of reactive oxygen species (ROS) compared to non-hypoxic cells. The ROS, H_2O_2 is an important second messenger involved in cell signaling. Nevertheless, excess ROS can cause DNA damage, protein oxidation and lipid peroxidation ultimately leading to apoptosis. For this reason, redox regulation plays a crucial role in cell integrity. We have previously shown that annexin A2 interacts directly and reversibly with H_2O_2 leading to its inactivation. We have also shown that annexin A2 antioxidant

function plays a crucial role in supporting tumor growth and chemoresistance. With this work we investigated if annexin A2 plays a role in the redox regulation of cancer cells during hypoxia. Material and Methods. In order to achieve our goal, we subjected annexin A2 shRNA and control (scramble shRNA) cancer cells to chemical hypoxia (200 μM CoCl2) or real hypoxia (1% O2) for different times and analyzed the activation of hypoxia induced signaling pathways by western blotting. We used 2',7' dichlorodihydrofluorescein diacetate (DCF-DA) reagent to study the intracellular levels of ROS in hypoxic annexin A2 depleted versus control cancer cells. To investigate the redox status of annexin A2 during hypoxia we performed BIAM (biotinylated iodoacetamide) assays. Results and discussion. Our results showed that annexin A2 redox status changes during hypoxia, and that annexin A2 depleted cancer cells accumulate higher levels of ROS under hypoxic conditions compared to control cells. These results indicate that annexin A2 antioxidant function regulates the ROS levels of cancer cells during hypoxia. It is well established that in addition to low levels of oxygen, ROS play a key role in HIF-1α stabilization. HIF-1α translocates to the nucleus where it dimerizes with HIF-1β and associates with co-activators such as CBP/p300 leading to the activation of more than one hundred genes that are crucial for the adaptation of cells to hypoxic stress. We observed that hypoxic annexin A2 depleted cancer cells show higher levels of hypoxia inducible factor 1 alpha (HIF-1α) and increased activation of HIF dependent signaling pathways compared to control cells, which is concomitant with the increased levels of ROS observed in these cells.

No conflict of interest

C11. GPER is differentially expressed in prostate cancer cell lines

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Introduction: G protein-coupled estrogen receptor (GPER), also known as GPR30, belongs to the seven transmembrane receptor superfamily and is involved in the rapid nongenomic estrogenic responses. Although the development and progression of prostate cancer (PCa) are highly dependent on hormonal actions, and recent work of our research group have indicated that estrogens may be protective agents in prostate carcinogenesis, the physiological effects of estrogens in PCa have been mainly associated with the differentially activation of classical nuclear estrogens receptors (ER). Nevertheless, GPER actions have been linked to antiproliferative and proapoptotic effects, so it is likely that GPER mediates the "anti-carcinogenic" actions of estrogens. As a first step to disclose the role of GPER in PCa, this work aims to characterize the GPER expression and its subcellular localization in non-neoplastic prostate cell and in cell line models of PCa representing different stages of the disease. Materials and Methods: The non-neoplastic cell line PNT1A and the neoplastic cell lines LNCaP, DU145 and PC3 were maintained in culture in RPMI 1640 medium under standard conditions. GPER protein expression in neoplastic and non-neoplastic cell lines was quantified by means of Western Blot technique. Moreover, cultured cells were fixed with 4% PFA and permeabilized with 1% Triton X-100 for fluorescent immunocytochemistry and determination of the subcellular localization of GPER. The GPER location at cell membrane, endoplasmic reticulum and nucleus was evaluated by colocalization with wheat germ agglutinin, calnexin and hoescht, respectively. Results and Discussion: A differential expression of GPER in prostate cell lines depending on the disease status was found. GPER expression was highest in less aggressive androgen-dependent LNCaP cell and decreased in more aggressive castration-resistant cell line models (DU145 and PC3). Moreover, GPER was located at the cell membrane and endoplasmic reticulum, and also in the nucleus. The downregulated expression with the aggressiveness of PCa and the subcellular location of GPER are supportive of its beneficial role restricting proliferation and inducing apoptosis. These preliminary results are encouraging to explore further the GPER role in prostate carcinogenesis and its potential as therapeutic target. Keywords: GPER, GPR30, PNT1A, LNCaP, DU145 and PC3.

No conflict of interest

D1. TRIB2-mediated AKT activation provides melanoma cells with a novel regulatory mechanism underlying drug 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 to treat melanoma or which are currently tested in clinical trials. Importantly we show that this novel mechanism of drug resistance has clinical relevance Materials and Methods: Using paired isogenic cell lines harboring silenced or overexppresed TRIB2, 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. 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 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 proposal for clinical management of melanoma patients are extremely important. Keywords: Melanoma, drug resistance, FOXO, AKT, PI3K p53.

No conflict of interest

D2. POLE somatic mutations in advanced colorectal cancer

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Introduction: Despite all the knowledge already gathered, the picture of somatic genetic changes in colorectal tumorigenesis is far from complete. Recently, germline and somatic mutations in the exonuclease domain of polymerase, epsilon, catalytic subunit (POLE) gene have been reported in a small subset of microsatellite stable and hypermutated colorectal carcinomas (CRC), affecting the proofreading activity of the enzyme and leading to misincorporation of bases during DNA replication. Materials and Methods in order to evaluate the role of POLE mutations in colorectal carcinogenesis, namely in advanced CRC, we searched for somatic mutations by Sanger sequencing in tumor DNA samples from 307 cases. Microsatellite instability and mutation analyses of a panel of oncogenes were performed in the tumors harboring POLE mutations. Results and Discussion Three heterozygous mutations were found in two tumors, the c.857C>G, p.Pro286Arg, the c.901G>A, p.Asp301Asn, and the c.1376C>T, p.Ser459Phe. Of the POLE mutated CRC, one tumor was microsatellite stable and the other had low microsatellite instability, whereas KRAS and PIK3CA mutations were found in one tumor each. We conclude that POLE somatic mutations exist but are rare in advanced CRC, with further larger studies being necessary to evaluate its biological and clinical implications.

No conflict of interest

E1. TRIB2 expression correlates with worse prognosis in cancer patients

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Introduction: The emergence of drug-resistant tumour cells is a major obstacle for both conventional chemotherapeutics as well as novel targeted therapeutics. Critical transcription factors such as forkhead box O (FOXO) proteins have been shown to mediate the action of multiple anti-cancer drugs, including PI3K pathway inhibitors. As FOXO proteins play a key role in the action of several anticancer drugs, proteins that are capable of suppressing FOXO activity are strong candidates to confer drug resistance. Our large-scale genetic screen identified the kinase-like protein Tribbles 2 (TRIB2) as a FOXO suppressor protein while TRIB2 has also been implicated in the development and progression of melanoma and leukemia. In this study we show that tumours that overexpress TRIB2 are resistant to in vivo pharmacological PI3K inhibiton. Moreover, TRIB2 expression is significantly increased in tumour tissues from patients with primary melanoma, colon and pancreatic cancer. Materials and Methods: Surgically excised tumour tissue samples from colon cancer primaries, pancreatic cancer primaries, and melanoma metastases were obtained from patients prior to first-line systemic therapy. Colon carcinoma and pancreatic tumour tissue (and matched normal tissue) was obtained during surgery. Melanoma tumour samples were obtained from metastatic lesions from patients with advanced melanoma in AJCC stage IV. The samples were freshly frozen and cryo-preserved until processing. The clinical data of the corresponding patients were extracted from the patient files. We performed quantitative RT-PCR to evaluate TRIB2 expression levels in tumour samples. Additionally, we used western-blot to assess TRIB2 protein levels as well activation status of core components of the PI3K-AKT pathway. Results and Discussion: Compared to normal tissue samples, TRIB2 transcription and TRIB2 protein expression was significantly increased in metastatic melanoma, primary colon and primary pancreatic cancer tissue samples. Concomitant with TRIB2 status, we found that pSer473-AKT1 and pSer253-FOXO3a protein levels were significantly higher, while the transcript and protein levels of the FOXO-dependent genes were significantly lower in ex vivo melanoma samples compared to normal control tissue samples. Finally, we examined the clinical significance of our ex vivo data within large patient cohorts. For each cancer entity, high TRIB2 expression correlated with a significantly worse clinical outcome. Overall, our data suggests that TRIB2 is a suitable biomarker predicting treatment outcome and selecting patients for individualized therapy.

No conflict of interest

E2. Actionable biomarkers CDX2 and SOX2 predict treatment response in colon cancer

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Introduction: Colorectal cancer (CRC) is one of the most common human malignancies. Tumour staging provides the basis for treatment decisions and remains the major prognostic marker. CDX2 and SOX2 are transcription factors associated with differentiation and stemness, respectively. Moreover, SOX2 has been widely related to tumor aggressive features in diverse models, namely recurrence and metastasis. We sought to explore the impact of CDX2 and SOX2 expression in patient prognosis and treatment response. Materials and Methods: CDX2 and SOX2 expression were assessed in a TMA comprising 903 colorectal tumours and crossed with clinicopathological, treatment and follow-up data to determine their association with tumor behavior and patient outcome. Moreover, we used the LS174T WT cell line and two CDX2-knock-out isogenic clones to study CDX2/SOX2 impact, with or without SOX2 inhibition with siRNAs, on 5-fluorouracil-induced apoptosis, evaluated by flow cytometry following Annexin V/PI staining. Results and Discussion: CDX2 was strongly expressed in 93.5% (586/627) of the tumours and significantly associated to better patient overall survival (p=0.007), appearing as an independent prognostic marker in multivariate analysis (OR=0.697, p=0.009). A cellular model with CDX2 knock-out showed increased resistance to 5-FU-induced apoptosis. Interestingly, these knock-out cell lines present with a great increase in SOX2 expression, commonly associated to higher aggressiveness features, namely resistance to therapy. SOX2 downregulation validated its involvement in the chemoresistance of the clones. Remarkably, SOX2 and CDX2 also appeared inversely correlated in the patient series (p<0.001) and associated to inverse tumour features regarding location, histological grade and MSI status. Although SOX2 expression did not predict overall survival with statistical significance, within stage III patients subjected to chemotherapy, SOX2 expression, considered alone or in combination with CDX2, was strongly associated with worse patient outcome (p=0.005), in line with our in vitro findings. Altogether, we put forward SOX2 as a biomarker of poor prognosis which may be used to stratify stage III colorectal cancer patients into subgroups with significantly different therapy response and outcomes.

No conflict of interest

E3. ΔNp63 is critical for progression of high grade non-muscle invasive bladder cancer through deregulation of specific genetic path

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Introduction: High Grade Non-Muscle Invasive Bladder Cancer (HG-NMIBC) represents a heterogeneous disease with very different outcomes depending on its progression to muscle invasive disease (MID). Due to its high recurrence rate, it presents prominent tumor-related morbidity and it is one of the costliest cancers in terms of overall expenditures. Even though some clinical and molecular risk factors have been reported to predict progression, they have shown limited prognostic ability in the clinical setting. Thus, it is critical to identify markers that can help clinicians provide individualized risk-stratified decision-making. We have previously reported the significance of ΔNp63 as a protective individual marker of HG-NMIBC tumor progression. The main goal of this project was to study the mechanism by which ΔNp63 loss triggers tumor progression to MID. Material and methods. We generated ΔNp63 Knock-down cell lines (shΔ) using specific shRNA against the ΔNp63 isoform in RT112 and BFTC cell lines. Gene expression and in vitro functional analysis such as cell cycle, proliferation, colony formation and matrigel invasion assays were performed. Moreover, we used a bladder orthotopic xenograft mouse model to examine the in vivo effects of ΔNp63 loss. Results and discussion. We observed that RT112_shΔ and BFTC_shΔ cells showed higher proliferation rate both in vitro and in vivo as well as increased colony formation activity when compared to parental cells. Furthermore, they displayed superior invasion capacity in vitro, which correlated with higher local tumor initiation and metastatic potential in the in vivo model. Gene expression analyses revealed that 9 significantly upregulated and 4 downregulated genes were common between both experimental cell lines. Functional studies to assess which genetic pathways are critical for tumor progression subsequent to ΔNp63 loss are further being pursued. Our

findings could ultimately assist clinicians in developing personalized medicine, identifying high-risk patients who could benefit from early radical cystectomy to prevent tumor progression, as well as low-risk patients who could benefit from more spaced follow-up visits. Consequently, this could have a great impact in reducing the economic cost of this disease. Finally, these studies may unlock the opportunity of identifying new therapeutic strategies to target previously understudied pathways in HG-NMIBC, therefore avoiding progression to more aggressive disease.

No conflict of interest

E4. Inhibition of mast cells' degranulation on mammary carcinogenesis

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Introduction: Mast cells are among the non-malignant cells that constitute tumor microenvironment. Once the mast cells in breast cancer have been associated with tumor aggressiveness and poor prognosis in women and female dogs, a hypothesis arises that the inhibition of their degranulation might suppress tumors' growth. In this way, this work aimed to evaluate the effects of mast cells' degranulation inhibition with ketotifen on mammary tumors chemically-induced in female rats. Materials and Methods: Twenty female Sprague-Dawley rats with four weeks of age were randomly divided into two experimental groups: N-methyl-N-nitrosourea (MNU) (n=10) and MNU+ketotifen (n=10). At seven weeks of age, all animals received an intraperitoneal injection of MNU (50 mg/kg). Animals from MNU+ketotifen group received ketotifen immediately after MNU administration in drinking water at a concentration of 1 mg/kg, 7 days/week for 18 weeks. Animals from MNU group received only water during the protocol. After this, all surviving animals were sacrificed. Mammary tumors were histologically evaluated by a pathologist. All procedures followed the National and European legislation on the protection of animals used for scientific purposes and were approved by National (Approval no. 008961) and University (CE_12-2013) Ethics Committees. Results and Discussion: One animal from MNU+ketotifen group died during the experiment. Six animals (60%) from group MNU and eight animals (89%) from group MNU+ketotifen developed mammary tumors. At the end of the protocol, a total of 35 and 44 mammary lesions were identified in MNU and MNU+ketotifen groups, respectively. The majority of lesions were classified as malignant (33 in MNU group and 40 in MNU+ketotifen group). Papillary non-invasive carcinoma was the lesion most frequently identified in both groups. Inversely to that expected, the inhibition of mast cells' degranulation did not change the mammary tumors' progression. Funding: This work was supported by European Investment Funds by FEDER/COMPETE/POCI - Operational Competitiveness and Internationalization Program, under Project POCI-01-0145-FEDER-006958 and Portuguese Foundation for Science and Technology (FCT), under the project UID/AGR/04033/2013, the project PTDC/DES/114122/2009 and post-graduation grant SFRH/BD/102099/2014.

No conflict of interest

E5. P-cadherin: a candidate biomarker for axillary-based clinical decisions in locally advanced breast cancer

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Introduction: Breast cancer is the most frequent cancer in women worldwide and a major cause of morbidity and mortality. In general, metastatic spread is the major reason behind the fatal outcome. According to the Guidelines of ESMO 2015, the presence of axillary lymph node metastases (N) is one of the most important prognostic factors in breast cancer, a cornerstone to clinical practice decisions. For example, the clinical decision tool Adjuvant Online considers the number of positive nodes as a crucial item for the verdict concerning therapy after surgery. However, the molecular biology related with the cancer cells growing in the lymph nodes is almost not taken into account to clinical decisions, mainly due to the assumption that their positivity would be the same that the one scored at the primary tumour. There is a lack of specific axillary biomarkers to predict patient prognosis when the N is positive. Based on the cancer stem cell (CSC) concept, we hypothesised that CSC markers could be good candidates to be tested as biomarkers in axillary metastases, in order to evaluate, at diagnosis, how patient's prognosis will be. Methods: We studied by IHC methods the expression of P-cadherin, CD44 and CD49f, already associated to stem cell properties in breast cancer, in a series of 135 primary tumours and synchronous axillary lymph node metastases Results: We found that P-cadherin expression in lymph node metastases was significantly associated with poor patient's overall and disease-free survival. Although the majority of the cases have shown a concordant result between the matched cases, we found some discrepancies. P-cadherin and CD49f were enriched in the lymph nodes when the matched primary tumours were negative, mainly found within the triple negative molecular subtype. Importantly, we still found that cases that gained P-cadherin expression in lymph node metastasis presented the worst survival endpoints of the whole series, as well as the ones that lost its expression in the lymph nodes, showed a good prognosis besides the positive expression at the primary tumour. Discussion: P-cadherin is an important predictor of disease outcome of breast carcinomas with lymph node involvement, being a valuable marker for disease progression surrogate end points. These results should put in perspective, that the term "positive axilla" is actually very deceptive just considering the observation of cancer cells and biomarkers urge for clinical decisions in this field.

No conflict of interest

E6. Assessing drug efficacy in co-cultures of Non-Small Cell Lung Carcinoma cells with fibroblasts in stirred-tank culture systems

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Introduction: Co-cultures of tumor cells with fibroblasts are currently highly coveted in cancer research to assess the role of cross-talk between these two cell types in tumor progression and acquisition of drug resistance. Numerous 3D tumor cell models have been proposed in recent years aiming to reflect the high complexity of the tumor microenvironment. However, most of these models do not allow long-term culture, continuous monitoring and often use bioactive scaffolds. Materials and Methods: We have developed a novel model system for the long term co-culture of tumor and fibroblasts to assess tumor-stoma cross-talk. Our strategy is based on stirred-tank culture systems and alginate microencapsulation. Non-Small Cell Lung Carcinoma (NSCLC) tumor cell aggregates (H1650 and H1437 cells) were microencapsulated in alginate alone or in combination with fibroblasts (immortalized normal and cancer-associated fibroblasts – NFs and CAFs, and human dermal fibroblasts - hDFs) and cultured for up to 20 days. The effect of the presence of fibroblasts was assessed by analysis of tumor growth and drug response, both in vitro and in vivo (lung orthotopic or subcutaneous implantation in mice). Co-cultures were challenged with standard of care drugs: docetaxel as

chemotherapeutic drug, and erlotinib and GSK1059615 (dual mTOR/Pl3k inhibitor) as targeted therapies. Cell proliferation, viability and aggregate diameter were analyzed as read-outs of drug efficacy. Results and Discussion: Microencapsulation of H1650 and H1437 spheroids with fibroblasts resulted in viable cell cultures, with tumor aggregate area increasing continuously during culture time, both in mono- and co-cultures. In vitro co-culture of H1437 cells and hDFs resulted in a statistically significant tumor cell growth, which was also confirmed by lung orthotopic implantation, leading to increased tumor load and accelerated mortality in comparison with H1437 mono-cultures and co-cultures with other sources of fibroblasts. On the other hand, co-cultures of H1650 with fibroblasts did not lead to significant changes in tumor cell growth, both in vitro and in vivo. GSK1059615 and docetaxel treatments in H1437 mono- and co-cultures resulted in increased cell death and decreased proliferation in both conditions, with no significant differences. Treatment of H1650 mono- and co-cultures with docetaxel and erlotinib led to delayed tumor growth rate, both in vitro and in vivo, with no significant impact of co-cultures on tumor sensitivity to these drugs. In summary, we demonstrated that our robust model system for long-term in vitro recapitulation of tumor-stroma crosstalk is applicable to drug screening, both in vitro and in vivo. These results suggest that the effect of tumor-stroma co-cultures is dependent on the tumor cell-fibroblast pair. Acknowledgements: We acknowledge Dr. H.v.d. Kuip and Dr. M. Oren for the supply of the cell lines. This research received support from the Innovative Medicines Initiative Joint Undertaking (grant agreement n° 115188), FCT (iNOVA4Health—UID/Multi/04462/2013). MFE and SA are recipients of PhD fellowships from FCT (SFRH/BD/52208/2013 and PD/BD/105768/2014).

No conflict of interest

E7. P hosphorylated YB-1 (Y-box binding protein 1) as a biomarker of poor prognosis in patients with breast cancer

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Introduction: YB-1 (Y-box binding protein 1) is a multifunctional cold-shock protein constitutively expressed in tissues. YB-1 plays a major role in most cellular functions and has been implicated in all hallmarks of cancer. Elevated YB-1 expression has been correlated with cancer progression and poor prognosis in several types of cancers, including breast cancer (BC), where high YB-1 expression seems to be related to lower overall survival (OS) and distant metastasis free-survival (DMSF) across all subtypes. The phosphorylated form of YB-1 (p-YB-1) seems to be involved in the transcriptional regulation of important genes like EGFR and ERBB2, but its role as a biomarker is unknown. In this study we assessed the prognostic value of p-YB-1 at diagnosis or relapse, in patients with advanced BC. Methods: In this retrospective study we included 65 patients diagnosed with BC, either metastatic at diagnosis or that relapsed at distant sites on the course of the disease. Expression of p-YB-1 was evaluated by immunohistochemistry in 65 primary tumors and 32 paired metastases, using an anti-human p-YB-1 (Ser102) antibody (Cell Signaling), and the EnVision Detection System (Dako). Negative control was obtained by pre-treatment with Calf alkaline phosphatase. Staining intensity was classified from 0 to 3, and samples were scored according to the percentage of cells with a cytoplasmatic staining intensity ≥2, and the positivity or percentage of cells with nuclear staining for p-YB-1. Cytoplasmatic p-YB-1 expression was considered high at a cut-off of 35 or 20%, for primary tumors or metastases, respectively, as determined using Cutoff Finder 2.1 (Institute of Pathology, Charité - Universitätsmedizin Berlin) and OS as endpoint. Differences in clinicopathological characteristics were tested using Fisher exact test. Time to event outcomes were analysed using Cox models. Results and Discussion: High cytoplasmatic p-YB-1 expression was found in 23% of primary tumors and 32.3% of metastases. In primary tumors, high p-YB-1 was associated with ER/PR negativity (P=0.006 and P=0.037, respectively), and with lower DMFS (P=0.0108, HR 0.3058 95%CI 0.1230-0.7606), but not significantly with OS (P=0.0687, HR 0.4789 95%CI 0.2168-1.058). Among metastases, high p-YB-1 was correlated with PR negativity (P=0.030). In this setting, high cytoplasmatic p-YB-1 was prognostic of decreased OS (P=0.009, HR 0.2394 95%CI 0.08186-0.7002). Nuclear expression of p-YB-1 was not indicative of prognosis. Therefore, p-YB-1 may be important an important prognostic biomarker of relapse in patients with BC, and of decreased OS in patients with metastatic disease, especially in patients with ER-/PR- tumors.

No conflict of interest

E8. Contribution of MUTYH germline mutations to early onset non-polyposis colorectal cancer

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Introduction: The incidence of colorectal cancer (CRC) in young patients (diagnosed at age ≤50), with or without a weak family history of CRC, is increasing and, in the majority of cases, the underlying genetic causes for CRC susceptibility remain unclear. These patients are suspected of Lynch syndrome and should undergo microsatellite instability (MSI) testing and/or immunohistochemical analysis of mismatch repair (MMR) proteins. Some studies have shown that mutations in genes associated with hereditary syndromes, like MUTYH, may explain a small percentage of CRC at young age. We aimed to evaluate the contribution of MUTYH germline mutations to early onset non-polyposis CRC from our Familial Cancer Registry. Materials and methods: We performed MUTYH mutation analysis using next-generation sequencing and MLPA. MSI and MMR immunohistochemical analysis were performed whenever tumor was available. Statistical analysis: STATA 12. Results and discussion: So far, MUTYH mutation analysis was performed in 38 patients (16 male:22 female; mean age: 40, 23-50). Clinical features: CRC localization (proximal-9; distal-12; rectum-13); 41% of the patients presented adenomas (1-5), 3 cases only with serrated adenomas; 12% and 21% of the patients presented familial history of CRC or adenomas, respectively; 21% showed familial history of other neoplasias. 13% of CRC were MSI-high, 19% MSI-low and 68% MSS. Amongst CRC showing MSI-H, all showed absence of MLH1/PMS2 expression (4/4). No MUTYH biallelic mutations were found. MUTYH mono-allelic mutations were detected in 2/38 (5%) patients, in both cases the hotspot G396D. This is in agreement with the higher frequency of G396D relatively to the other hotspot (Y179C) in the Iberian Population. The age at CRC diagnosis in these two patients was 37 and 39, respectively, and both tumours were MSS. The former patient presented 4 serrated adenomas and a family history of adenomas whereas the latter had no personal or family history of adenomas. Our results suggest that biallelic MUTYH mutations are relatively rare in young (≤50) non-polyposis CRC patients from Southern Portugal. On the other hand, we observed a high prevalence of monoallelic MUTYH mutations in our cohort, which may result from the small patient's group. However, one cannot exclude, in agreement with previously reports, a modestly increased risk for the development of adenomas and cancer in monoallelic carriers.

No conflict of interest

E9. Microenvironment modulation in renal cell carcinoma by extracellular miR-210 and miR-1233: implications in disease aggressiveness

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Introduction: Renal cell carcinoma (RCC) is the most common solid cancer of the adult kidney, accounting for approximately 90% of kidney cancers, with the incidence and mortality rate increasing by 2-3% per decade. This reality and the nonexistence of a standard screening test, contributes to the fact that one-third of patients are diagnosed with local invasive or metastatic disease. Moreover 20-40% of RCC patient's submitted to surgical nephrectomy will also develop metastasis. The hypothesis of molecular signaling between cells has been revolutionizing molecular biology and giving space for reformulating the hypothesis of tumor microenvironment as modulator of the metastatic process. Among the molecules that can be released from

cells we can find microRNAs (miRNAs). MiRNAs are small non-coding RNAs that are responsible for the regulation of numerous genes at a post-transcriptional level. MiRNAs have been widely studied in oncology since they have been proved to be deregulated in cancer and also have influence in cancer development. The aim of this study was the establishment of a circulating miRNA expression profile that could be used as a biomarker of diagnosis and/or prognosis in RCC patients. First, we performed an in vitro study using renal cell lines, one normal immortalized and the other metastatic (HK-C8 and FG-2, respectively), to establish a profile of deregulated miRNAs in RCC. In a second phase, we validated the miRNA profile in plasma samples of RCC patients and healthy individuals, and evaluated its impact in the patients' time to progression. Material and Methods: The miRNA expression was analyzed by quantitative real-time PCR in both cell lines (medium vs cells) and in our study population (n=50 healthy individual's vs n=50 RCC patients). The 2-ΔΔCt method, along with the t' Student test were used in order to evaluate statistical differences in the levels of the miRNAs. The Kaplan-Meier method and Log-rank test were used to compare the miRNA profile influence in time to disease progression. Results and Discussion: miR-210 and miR-1233 were overexpressed in FG-2 RCC cell line when compared to the immortalized normal renal cell line (HK-C8). We also observed an increase in the levels of these miRNAs in plasma samples of RCC patients and its association with higher tumor size (miR-210, P≤ 0.001; miR-1233, P = 0.011), higher Fuhrman nuclear grade (miR-1233, P = 0.011) and presence of metastasis at the time of diagnosis (miR-210, P = 0.010; miR-1233, P≤ 0.001). Additionally, we observed that patients presenting higher levels of both miR-210 and miR-1233 had a lower time to progression (Log Rank test, P =0.015; HR = 3.22, 95%CI 1.01 – 10.26, P = 0.048). Based on these results, we consider that miR-210 and miR-1233 are good candidates for biomarkers of prognosis and aggressiveness in RCC.

No conflict of interest

E10. Out of the bag – functional characterization of ex vivo expanded MSC

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Introduction: Bone marrow (BM) derived mononuclear cells (MNC) are the starting point of numerous protocols, such as ex vivo expansion of HPC, neuro and mesenchymal stem cells (MSC). We have previously established that the BM collection bag and filter system (which are usually discarded) are an alternative source of MNC, yielding viable and sterile cells equivalent to 50ml of filtered BM, and should not be considered clinical waste. The viability and functionality of the recovered MNC were evaluated by trypan blue exclusion and in vitro differentiation into MSC, respectively. In order to further validate the applicability of these cells, MSC obtained were tested for morphology, immunophenotype, ability to hamper alloreactivity and to differentiate into adipocytes. Materials and Methods The collection bag and filter system were back-washed and rinsed with RPMI under aseptic conditions and MNC isolated by density gradient centrifugation. To obtain long term MSC cultures, MNC were cultured in DMEM media supplemented with foetal bovine serum, at 37ºC and 5% CO2. Cultures were replated when confluence was reached. MSC morphology was confirmed with a reverse microscope, and immunophenotype by flow cytometry. To assess adipogenic differentiation capacity, MSC were cultured in the presence of dexamethasone and diclofenac, with adipocytes generation tested by Oil Red staining of lipid deposits. To evaluate their imunossupressive role a one way MLR was performed. Briefly, irradiated MNC (stimulator) and CFSE-dyed MNC (responder) were co-cultured in the presence or absence (control) of MSC. After 7 days of incubation, proliferation of responder cells was measured by flow cytometry Results and Discussion In all cases, filter recovered MNC viability was superior to 90%, and long term MSC cultures were established, as shown by morphology and immunophenotype (CD105+, CD44+, CD90+, CD73+, CD45- , CD14-, and CD34-). To further characterize our MSC lineage differentiation, a hallmark of MSC, was shown by Oil Red staining of MSC-derived adipocytes. Another characteristic of MSC, down regulation of alloreactivity, was demonstrated in vitro, with a consistent 20% reduction of proliferating cells. With this study we demonstrated that, from the usually discarded BM collection bag and filter system, long term MSC cultures can be established. These cells can be expanded ex vivo, maintaining an appropriate phenotype and functional capabilities, being suitable for both investigation and clinical settings.

No conflict of interest

E11. PIK3CA mutant allele differential expression (MADE) association analysis with breast cancer

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Introduction: Cis-regulatory variation is responsible for differential allelic expression (DAE), a common characteristic of autosomal genes, and is pivotal in determining intra-species phenotypic individuality but its effect on tumour biology is largely unexplored. We hypothesise that it may contribute to tumour biology, by generating imbalanced expression between wild-type and mutant alleles of cancer driver genes. Materials and Methods We investigated the existence of DAE in normal breast tissue for the PIK3CA/AKT1 pathways genes, using data from single-nucleotide polymorphism (SNP) arrays performed on DNA and RNA for a set of 64 normal breast tissues. We also mapped the cis-regulatory variation contributing to the DAE displayed by the gene PIK3CA in normal breast tissue. Next, we calculated the mutant by wild-type (mut/wt) allelic ratios in the RNA, with and without correction for copy number (achieved by normalising with the mut/wt allelic ratio observed in the DNA). We performed this in a series of 42 breast tumours, with clinical data, for which we had DNA and RNA available. Results and Discussion We found that DAE is frequent in the genes involved in the PIK3CA/AKT1 pathway, the most mutated pathway in breast cancer. We have also identified a single SNP, rs2699887, located at the promoter of PIK3CA which is associated with DAE in the gene PIK3CA (permuted p-value=0.04), in normal breast tissue. We have detected differential allelic protein binding for this SNP in vitro. We are now finishing functional analysis to dissect the mechanism responsible for DAE. Interestingly, in breast tumours, we found that MADE was evident in PIK3CA, even after correcting for copy number in DNA. Our cancer cohort is currently too small to investigate significant correlation with clinical variables. Therefore, we are extending our study to include an extra 100 breast tumours, harbouring PIK3CA missense mutations. We show that the expression of genes coding for a large proportion of the PIK3CA/AKT1 pathway components are regulated by cis-regulatory variation, that in the case of PIK3CA could be due to a single causal SNP in the promoter. With this work, we will establish whether MADE, mutant allele differential expression, plays a role in breast tumour clinical characteristics. Further exploration of this mechanism in other genes belonging to this pathway will allow a better understanding of tumour development, and contribute to better patient management.

No conflict of interest

E12. STAT5 activation associates with high risk and poor prognosis in pediatric B-cell acute lymphoblastic leukemia

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Introduction: Acute lymphoblastic leukemia (ALL) is the most frequent childhood malignancy. Despite significant improvements in treatment outcome, around 10-20% of patients still relapse, so there is a clear demand for new prognostic factors predicting therapy response. Both cell-autonomous lesions and microenvironmental cues, such as IL-7, contribute to the activation of the pro-survival JAK-STAT5 pathway in ALL. Moreover, STAT5 is constitutively activated in Philadelphia (Ph)-positive B-cell ALL (B-ALL) cases, downstream of the BCR-ABL translocation, and its high phosphorylation has been proposed as a surrogate marker for the BCR-ABL1- like ALL subgroup, characterized by poor prognosis. However, it remains to be determined whether the STAT5 activation status has prognostic value in ALL. We propose to tackle this issue using phospho-flow cytometry, which is potentially applicable to clinical diagnostics. Materials and Methods: Bone marrow samples were collected at IPOL from pediatric B-ALL patients at diagnosis (n=86), after ethical approval and informed consent. STAT5 activation was determined by measuring the levels of Y694 phosphorylation (P-STAT5), both ex vivo and after stimulation with IL-7 in vitro, at the single-cell level, by using

phospho-flow cytometry. Values were normalized to those of a reference cell line (NALM6) and compared with parameters such as age, EGIL maturation stage, white blood cell count (WBC), minimal residual disease (MRD) at the end of induction therapy, relapse and event-free survival (EFS). Statistical analysis was performed using Kolmogorov-Smirnov or Kruskal-Wallis tests, as appropriate. P values < 0.05 were considered significant. Results and Discussion: Basal or IL-7-stimulated levels of STAT5 activation did not correlate with age or MRD. However, patient samples with a B-II ALL maturation stage were more responsive to IL-7 stimulation, as measured by increased P-STAT5 and cell size, than more immature (B-I) or more differentiated (B-III/IV) cases. This suggests that, in contrast to what happens in T-cell ALL, IL-7 differentially impacts on B- ALL depending on the developmental stage in which the leukemia cells are blocked. Most importantly, we found that higher ex vivo P-STAT5 levels associated both with higher WBC and with relapse. These observations are the first indicating that STAT5 activation may be a prognostic marker in B-ALL.

No conflict of interest

E13. Role of estrogen receptors in bladder cancer: preliminary results

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Introduction: As bladder cancer (BlaCa) has greater incidence among men but is more aggressive in females along with the discovery of estrogen receptors (ERs) in a subtype of BlaCa evidenced the need to understand the role of estrogens and their receptors in BlaCa development and progression. Furthermore, recent studies suggest that estrogen may have a role in urogenital schistosomiasis (UGS) and in schistosome-related BlaCa. This work aims to characterize the expression of ERα and ERβ in human BlaCa according to clinicopathological features. The effect of ERs agonists and antagonists in BlaCa cell proliferation were also evaluated using 3 human BlaCa cell lines: T24, HT1376 and 5637. Materials and methods: Tumor samples from patients with BlaCa treated at IPO-Porto (n=80) and patients infected with S. haematobium (n=62) from Angola, were evaluated by immunohistochemistry for the expression of ERα and β. Staining was classified according to the proportion and intensity of positive cells using the allred score. ERs expression in the cell lines was assessed by PCR and western blot. Response to treatment with ERs agonist (E2) and clinically approved antagonists (Tamoxifen, ICI 182 780) were evaluated by colorimetric cell viability assays and cell counting. The effect of the ERs disruptor in the relative expression of ERα were also evaluated through immunofluorescence imaging. Results and discussion: Nuclear expression of ERα was detected in 50% of the human bladder cancer samples from IPO in different cell types. Of those, 15% presented tumor cells staining, 19% had positive staining in the tumor stroma cells, 14% were positive in the adjacent urothelium (apparently normal) and 30% had positive staining in normal stroma cells. Regarding the samples from patients with UGS, 45% had reactivity, 19% in the tumor cells, 6% in the tumor stoma, 33% in the urothelium and 5% in the non tumoral stoma. The expression of ERα was associated with high grade tumors and poor survival in tumor patients from IPO and with the presence of schistosome eggs in the tissue samples from Angola. ERβ expression was detected in more than 85% of the cases in both series (IPO and Angola), mainly in tumor cells and urothelium. Staining intensity was higher in samples with UGS-related bladder cancer when compared to UGS alone. Both nuclear and cytoplasmic staining were observed. Regarding in vitro studies, T24 and 5637 cancer cells express ERα but not ERβ, while HT1376 cell line does not express ERs. In 5637 cells, E2 induced proliferation whereas the antagonists reduced cell survival representative of hormone-dependent (HD) growth. T24 cell survival was not affected by agonists, however it was inhibited by Tamoxifen and, to a lesser extent by ICI 182 780. This is characteristic of hormone-independent (HI) growth observed in progression of hormone-related diseases. Treatment with ICI decreased the expression of active ERα in 5637 cell line but not in T24 cells. Acknowledgements: this work was funded by Programa Operacional Factores de Competitividade COMPETE and National funds via FCT – Fundação para a Ciência e a Tecnologia under projectPTDC/SAU-ONC/118346/2010 (LAH) and PhD scholarship SFRH/BD/80855/2011 (CB).

No conflict of interest

F1. HDAC inhibition synergizes with Antioxidant therapy to target Myeloproliferative Neoplasms

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Introduction: The BCR-ABL-negative myeloproliferative neoplasms (MPN) are a group of heterogeneous hematological diseases with constitutive JAK-STAT pathway activation and Epigenetic alterations. However, despite the recent advances in unravelling disease etiology (JAK2 activating mutations), there is still no curative treatment outside bone marrow transplantation. Epigenetic alterations, like histone acetylation, play pivotal roles in the pathogenesis of hematological malignancies, and treatment of such disorders with histone deacetylase inhibitors (HDACis) results in cell death and growth arrest. Importantly, HDACis have proven to be effective in the clinical practice, by producing remissions and increasing the overall survival in blood malignancies. Moreover, in MPN patients HDAC inhibition has demonstrated some efficacy but it also presented toxicity. In order to explore the therapeutic potential of HDACis in MPN, we analyzed the effects of Vorinostat (an HDACi) on the biology of MPN cells. Material and Methods: MPN bone marrow samples were collected at diagnosis, the mononuclear cells were isolated and used for culture experiments. MPN cells, both primary samples and cell lines, were incubated with the different pharmacological reagents and at different time points, the cells were stained for Apoptosis and Reactive Oxygen Species (ROS) for detection by Flow Cytometry. Results and Discussion: Vorinostat decreased cellular viability primary MPN cells, particularly monocytes, associated with a concomitant decrease in ROS levels. In MPN cell lines we showed that a wide range of HDACi produced these effects. Interestingly, HDACi-induced apoptosis was dependent on decreased ROS levels suggesting that both events are connected and necessary for MPN cell death induced by HDACi to occur. By combining both HDACi and ROS reducing drugs (ROS production inhibitors; ROS scavengers and AntiOxidants) we were able to increase MPN cell death in a synergistic manner. AntiOxidant agents are easily accessible and this led us to hypothesize that an AntiOxidant-rich nutrition could benefit MPN patients that are undergoing Epigenetic treatments. These results point to a promising therapeutic strategy that needs to be confirmed in vivo and in the clinical setting.

No conflict of interest

F2. CCT241736 overcomes resistance to the clinical JAK2 inhibitor TG101348 through inhibition of JAK2/STAT3 signaling in pancreatic

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The acquisition of resistance to drugs is a common clinical problem for the treatment of many types of cancers. Pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating malignancy worldwide with an

extremely poor prognosis and the poorest survival rate among common cancers. The JAK2/STAT3 signaling pathway has been implicated in malignant transformation and development of PDAC. Several JAK2 inhibitors are currently being evaluated in the clinic for PDAC and development of resistance to these inhibitors has been reported that can result in reduced efficacy. The aim of our work was to evaluate whether our in-house Aurora kinase inhibitor CCT241736, which incidentally also inhibits JAK2, is able to overcome the acquired resistance in pancreatic cancer cells to JAK2 inhibitor, TG101348 currently undergoing clinical trials in many types of cancers. To achieve this, we developed a cell line resistant to TG101348 (CFPAC-1-Res) by continuous exposure to increasing concentrations of the inhibitor. The results demonstrated an activation of JAK2/STAT3 signaling pathway in CFPAC-1-RES cells when compared with parental cells (CFPAC-1-P; without exposure to TG101348), suggesting a possible mechanism of resistance to TG101348. Furthermore, CCT241736 was able to decrease JAK2/STAT3 pathway activation and to inhibit the growth of CFPAC-1-RES cells. We also found that the acquired resistance to TG101348 was not due to any mutations in the JAK2 catalytic domain, instead, possibly due, to the reactivation of the JAK2/STAT3 signaling. Our data suggests that CCT241736 may successfully overcome resistance to the JAK2-inhibitor TG101348 by decreasing JAK2/STAT3 signaling. This work proposes the benefit of a second line treatment with the in-house compound CCT241736 in resistance pancreatic cancer patients who undergo prolonged JAK2 therapy.

No conflict of interest

F3. Targeting Ewing Sarcoma Cells and the tumor microenvironment with OMTX003 Anti-Endoglin Monoclonal Antibodies

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Background: Ewing Sarcoma (ES) is a bone/soft tissue neoplasia affecting mainly children and young adults. ES is the second most common primary bone tumor after osteosarcoma in this age group. ES are heavily angiogenic and can show strong vascular mimicry. Endoglin (ENG) is a surface marker with an important role in the establishment of neo-angiogenesis and vascular mimicry. ENG is shedded from the cell surface by Matrix Metalloproteinase 14 (MMP-14) in its soluble form (sEDG) to the extracellular compartment. Materials & Methods: mRNA levels were evaluated by q-RT-PCR. Protein and subcellular location were studied by western blotting, immunofluorescence and flow cytometry in a set of cancer/non-tumoral cell lines. sENG in cell line supernatants were determined by ELISA. DNA promoter methylation study was performed in 9 ES cell lines and mesenchymal stem cells. Cytotoxicity assays with new human Monoclonal Antibodies (MAb) targeting Mouse ENG (mENG OMTX003) and Human ENG (hENG OMTX003) were performed by MTT assay and caspase 3 activation was evaluated by DEVDase activity. Xenograft models (n=30) with two ES cell lines were developed to study the in vivo tumor binding of the MAbs. A dose range study (DRS) was performed in healthy mice (n=15) exposed to 3 doses of hENG and mENG OMTX003 for 3 weeks. Results: Whole protein levels correlated with the mRNA levels, and the subcellular location of ENG was primarily at the cell membrane. Only one cell line (1/9) lacks expression of ENG, and in fact, this is the only cell line with hyper-methylated ENG promoter. The ratio between sENG/ENG in cell membrane showed a positive correlation with the levels of MMP14, consistent with a role of this protease in ENG shedding. Specific in vivo binding of hENG OMTX003 MAb after administration in xenografted mice of 2 ES cell lines (ENG++and ENG+/-) was observed in the ENG++ tumors. Impaired tubular formation during exposure to OMTX003 was observed in endothelial cells. Regarding the DRS, histopathological evaluation of several organs revealed no significant drug related toxicity after treatment with hENG OMTX003 up to 10mg/Kg and mENG OMTX003 up to 5mg/Kg. Conclusions: ENG is directly implicated in angiogenesis promoted by endothelial cells in the tumor microenvironment, and is also expressed in ES tumor cells. In vitro, OMTX003 inhibition of hENG reduces proliferation and impairs tubular formation of endothelial cells. In vivo, OMTX003 MAbs present specific binding to corresponding mENG/hENG and no toxicity was observed up to 10mg/kg dose. Efficacy of OMTX003 MAbs as anti-tumoral agents in mono/combined regimens is being currently assessed.

F4. Bioactive peptides as chemotherapeutic agents on metastatic breast cancer

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Introduction Advanced breast cancer is associated with the development of metastases in the bones, lungs, liver and brain and brain metastasis represent 15%-25% of the overall intracranial tumors. Despite all the efforts in anticancer drug development, new strategies are urgently needed for overcoming resistance and off-target effects of current therapies. Antimicrobial peptides (AMPs) represent a pool of novel templates for potential anticancer molecules. Their combination of decreased toxicity and resistance development with proven antitumor activities has directed research towards the study of the anticancer properties of these biologically active peptides. The human neutrophil peptide-1 (HNP-1) is an endogenous AMP pointed as a potential tumor biomarker. Previous work from our group revealed the preferential activity of this human defensin toward solid tumors when compared with a hematological cancer. PvD1 is a plant defensin isolated and purified from the seeds of common bean (Phaseolus vulgaris) with confirmed antifungal activity. This work reveals the effects of HNP-1 and PvD1 defensins on breast cancer cells and normal cells from breast and brain. Understanding the effects and mode of action of these anticancer peptides (ACPs) on breast healthy cells and on cells from a primary tumor will allow us to recognize potential drug leads for selective tumor targeting. In addition, observing the effects on human brain cells the development of new drugs for brain metastatic lesions treatment without significant damage to the human blood-brain-barrier can be improved. Materials and Methods The experimental approach of this work combined spectroscopic and atomic force microscopy (AFM) techniques for evaluating normal and tumor cells' viability and changes on the cellular membrane structure after peptides' interaction. The effects of both peptides on cell's biophysical and nanomechanical properties were followed by viability experiments with a tetrazolium compound, surface charge measurements and AFM (in imaging, single and cell-cell force spectroscopy modes). Results and Discussion Our results point to PvD1 selectivity toward cancer cells. The use of AFM reveals details of the cell membrane structure and morphology while surface charge studies show that cell death occurs without full neutralization of the tumor cell membrane. These changes at the cellular level deeply influence tumor cells' ability to migrate and invade the brain. Acknowledgements: This work was supported by a grant from Laço. D.G. acknowledges FCT-MEC for fellowship SFRH/BPD/73500/2010.

No conflict of interest

F5. Chromene-based new drug candidates for cancer treatment

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Over the last decade, significant improvements in the treatment of various cancers were achieved. Nevertheless, there is still an urgent need for novel, more potent and less toxic anti-cancer drugs as drug resistance and/or stability of the used drugs are still a problem, together with high toxicity and low tumor

selectivity. During the last years, it has become apparent that a number of natural products bearing the chromene scaffold have the capacity to prevent diseases. This family of compounds is widely present in edible plants and fruits and has encouraged medicinal chemists to explore their applicability for drug therapy. Many examples can be found possessing cancer preventive and anti-cancer properties. Several chromene-based compounds were synthesized bearing the 3-aminochromene unit as common feature, an important building block for new heterocyclic compounds. These compounds were prepared by simple and efficient experimental procedures starting from the reaction of 3-methoxysalcyladehyde and 1-cyanopyridinium chloride, leading to 2-imino and 2-oxo-2H-chromene. These chromenes, bearing the pyridine moiety in the C3 position, were deprotected using an efficiently Zincke-ring-opening reaction, allowing the isolation of the respective 3-aminochromene. These compounds were then further reacted with the appropriate reagents leading to substituted 3-aminocoumarines and chromeno[2,3-d] imidazole derivatives. An in vitro screening of the anticancer activity of the prepared chromenes was performed in three different cancer models: breast cancer (MCF7, MDA-MB 468, HS578t), glioblastoma (U87MG, GAMG, GL18) and leukemia (HL-60, KG-1, Jurkat). Cell viability was evaluated for all compounds using the MTS assay. The effect of these compounds will be further assessed, evaluating migration by the wound healing assay and cell death by caspase 3 and annexin V/PI assays. Additionally, toxicity of these compounds will be evaluated using a normal cell line (MCF10) and the C. elegans model, a popular and easy to handle platform in the field of the drug discovery. The chromeno[2,3-d] imidazole derivatives showed the most promising anticancer potential with IC50 values in the nano-Molar range. In conclusion, novel bioinspired compounds were synthesized by efficient synthetic approaches and the chromeno[2,3-d] imidazole derivatives demonstrated an interesting anticancer profile.

No conflict of interest

F6. The extracellular pH influences the cytotoxicity and transport of 3-Bromopyruvate in breast cancer cells.

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The anti-cancer properties of 3BP have been described previously, but its selectivity for cancer cells needs further explanation. We have characterized the kinetic parameters of radiolabelled 3BP uptake in three breast cancer cell lines with different levels of resistance to 3BP: ZR-75-1 < MCF-7 < SK-BR-3. At pH 6.0 the affinity of cancer cells for 3BP transport, correlates with their sensitivity, a pattern that does not occur at pH 7.4. The uptake of 3BP is dependent on the proton motive force and is decreased by MCTs inhibitors indicating MCT-1 as the major transporter. In the SK-BR-3 cell line, a sodium-dependent transport also occurs. Our results confirm the role of MCTs, especially MCT-1 in 3BP uptake and the importance of CD147 glycosylation in this process. We find that the affinity for 3BP transport is higher when the extracellular milieu is acid. This is a typical phenotype of tumor microenvironment and explains the lack of secondary effects of 3BP already described in in vivo studies. Acknowledgements This work was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through the COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI). João Azevedo-Silva received a fellowship from the Portuguese government from the FCT through FSE (Fundo Social Europeu) and POPH (Programa Operacional Potencial Humano) [grant number SFRH/BD/76038/2011]. This work was originally published in: Biochemical Journal 2015 Apr 15;467(2):247-58

No conflict of interest

F7. Modulation of membrane properties of lung cancer cells by azurin enhances the sensitivity to EGFR-targeted therapy and decreased

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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. In this work we demonstrate that azurin, an anticancer therapeutic protein originated from bacterial cells, 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, a tyrosine kinase inhibitor which targets specifically the EGFR, enhances the sensitivity of these lung cancer cells to this molecule. The broad effect of azurin at the cell surface level was examined by Atomic Force Microscopy. The Young 's module (E) shows 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. 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.

No conflict of interest

F8. ERCC1 A8092C (rs3212986) polymorphism: a prognostic value in cervical cancer?

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Introduction: In 2012, cervical cancer was the fourth most commonly diagnosed cancer in women and there were 528,000 new cases. Per day, it is estimated that several DNA-damaging events occur at a rate of 10,000 to 1,000,000 molecular lesions per cell. ERCC1 plays an essential role in the Nucleotide Excision Repair (NER) pathway and it is the main system to repair a wide variety of DNA damage, particularly bulky adducts, crosslinking and oxidative DNA damage. Due to the importance of genomic integrity maintenance, genes coding for DNA repair molecules have been proposed as candidatefor cancer-susceptibility genes. This gene is a more promising predictive biomarker of response to chemoradiotherapy, because its expression is associated with the repair mechanisms of ionizing radiation and cellular mechanisms of resistance to cisplatin. The aim of this study was to evaluate the influence of the ERCC1 A8092C polymorphism as prognostic marker for cervical cancer patients. Materials and Methods We analysed ERCC1 A8092C polymorphism genotypes in genomic DNA isolated from peripheral blood of 241 patients with cervical cancer who underwent a chemotherapy in combination with radiotherapy. Genotyping was performed by TaqmanTM allelic discrimination methodology. Kaplan-Meier method and Log-Rank test were used to obtain and analyse the survival curves. Results and Discussion The frequencies obtained for the AA, AC and CC genotypes were 10,4%, 32,3% and 57,3%, respectively. Concerning the overall survival rates found using Kaplan-Meier method, we found no association to the patients ERCC1 genotypes (p=0,739). Therefore, our results indicate that there is no any influence of the ERCC1 genetic variants on clinical outcome of cervical cancer patients. In literature, this polymorphism has been significantly associated with many neoplasias, like ovarian cancer, lung cancer, esophagus cancer. This is

the first study evaluating the role of the ERCC1 A8092C genetic variants in cancer prognosis and clinical outcomes of cervical cancer patients. Further functional studies regarding ERCC1 expression according to ERCC1 A8092C polymorphism genotypes should be conducted in order to validate this hypothesis.

No conflict of interest

F9. Possible influence of the P53 Arg72Pro polymorphism (rs1042522) in the clinical outcome of cervical cancer patients

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Introduction: Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases and 266,000 deaths worldwide in 2012. Currently, this malignant disease represents 7.5% of all female cancer deaths. DNA double-strand breaks (DSBs) are among the most cytotoxic DNA damages and failure to repair theses injuries results in genomic instability. The P53 gene is considered to be the guardian of the genome due to its role on cell cycle arrest, DNA repair activation and regulation of apoptosis. Given the functional relevance of the damage cellular response pathways on carcinogenesis, potential associations between genetic polymorphisms in genes involved in response of the cell to DNA damage, cancer risk and efficacy to therapy have been intensively evaluated. We conducted this study to show the possible influence of the P53 Arg72Pro polymorphism (rs1042522) in overall survival in cervical cancer patients. Material and methods Retrospective cohort study that includes 241 Caucasian patients with histological diagnosis of cervical cancer, FIGO stages IB2-IVA, treated with cisplatin-based chemotherapy and concomitant radiotherapy. P53 Arg72Pro polymorphism was analyzed by Taqman™ Allelic Discrimination methodology. The associations between this polymorphism and overall survival were estimated by Kaplan-Meier method and using Log Rank test. A p value < 0.05 was considered significant. Results and Discussion The P53 Arg72Pro polymorphism frequencies for homozygous Arg/Arg, heterozygous Arg/Pro and homozygous Pro/Pro were 0.56, 0.33, and 0.11, respectively. Our results demonstrate that the overall survival was statistically different according to the patients P53 Arg72Pro genotypes. The patients heterozygous Arg/Pro present a higher overall survival than patients homozygous Arg/Arg and Pro/Pro (135 months vs. 106 months; p = 0.043). These preliminary results may contribute towards a better understanding of the role of genetic polymorphisms in DNA damage response genes in treatment response in cervical cancer patients.

No conflict of interest

F10. A new prenylated chalcone acting as an antimitotic agent and inducing mitotic catastrophe

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Introduction Antimicrotubule agents are among the most effective chemotherapeutic drugs against many cancer types. Unfortunately, their use has showed limitations, associated with toxic side effects and drug resistance, thus stressing the need for novel antimitotic agents. One of the aims of research at "Laboratório de Química Orgânica e Farmacêutica da FFUP/CIIMAR" is concerned with discovery and obtaining of new small molecules, from natural and synthetic origin, with potential antitumor activity. From a library of compounds

emerged a synthetic prenylated chalcone, 2'-prenyloxy-3,4,4',5,6'-pentamethoxychalcone, reported to have potent anti-growth activity against different tumor cell lines (GI ≤ 10 μM). Here, we provide more insights into its biological activity. Materials and Methods Phase contrast microscopy and DAPI staining were used to evaluate the antimitotic activity of the compound in human breast adenocarcinoma MCF-7 cells. The effect on mitotic spindle was evaluated by anti-tubulin immunofluorescence, while activation of the spindle assembly checkpoint was assessed by Mad2- and BubR1-immunostaining. Cancer cell fate upon compound treatment was followed by live-cell imaging. Apoptosis was detected by TUNEL assay and fluorescence microscopy. Results and Discussion The compound induces the collapse of mitotic spindle and arrests cells in mitosis with activated spindle assembly checkpoint in MCF-7 cells. Live-cell imaging revealed that the compound induces mitotic catastrophe and massive cell death by apoptosis after a sustained delay in mitosis. These data suggest that anti-microtubule and antimitotic activities underlies the mechanism by which 2'-prenyloxy-3,4,4',5,6'-pentamethoxychalcone inhibits cancer cell proliferation, underscoring its antitumor potential.

No conflict of interest

F11. Anti-tumor efficacy of new aromatase inhibitors: androgen- and estrogen-receptors involvement on MCF-7aro cell proliferation

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Introduction: Breast cancer is the most common cause of cancer death in women worldwide, being the most prevalent the estrogen receptor-positive [ER+] breast tumors [1]. Estrogens are responsible for the development of ER+ breast tumors, thus, a treatment that blocks its action is an important mark for cancer therapy. One of the therapeutic strategies is the use of aromatase inhibitors (AIs) that inhibit the enzyme aromatase, which catalyzes the final step of estrogen's biosynthesis. Although, the AIs used in clinic proved to be effective, they cause some serious side effects including bone loss and the development of resistance. For this, the search for novel potent compounds, with fewer side effects, is currently needed. The present work focused on the study of the biological effects of four new steroidal compounds (57, 58, 59 and 60), which were designed and synthesized from structural modifications on the aromatase substrate androstenedione [2], and demonstrated to be potent AIs in human placental microsomes. Material and Methods: To evaluate their anti-tumor efficacy, it was studied the effects on viability of an ER+ human breast cancer cell line that overexpresses aromatase (MCF-7aro), by MTT assay. It was investigated if the in vitro effects were aromatase-dependent, using MTT assay and Western-Blot. It was also explored if these actions were androgen and/or estrogen receptors dependent, by using an androgen receptor (AR) antagonist and comparing the effects with an ER-negative breast cancer cell line (SK-BR-3). Results and discussion: All the AIs are capable of decreasing the viability of MCF-7aro cells in a dose- and time-dependent manner but independently of aromatase. Contrary to exemestane, these compounds did not induce aromatase degradation. Moreover, all the AIs caused anti-proliferative effects that are ER-dependent and AR-independent. These results suggest that these new AIs have anti-tumor properties in ER+ breast cancer cells. This work provides new information about on the most favorable structural modifications in androstenedione structure in order to design new and potent AIs with lower side effects. Acknowledgements: FCT: Amaral C. grant (SFRH/BPD/98304/2013) and (UID/MULTI/04378/2013 − POCI/01/0145/FERDER/007728); Prof. Shiuan Chen (Beckman Research Institute, USA) for MCF-7aro/LTEDaro cells. [1] Mohamed A., et al. (2013), Am J Pathol. 183(4):1096-112. [2] Amaral C., et al. (2013), J Steroid Biochem Mol Biol, 135:51-9.

No conflict of interest

F12. Chromene derivatives as anticancer agents: synthesis and biological evaluation

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Cancer is a heterogeneous and devastating disease worldwide, with millions of diagnosis per year and many people living with this pathology. This increasing cancer incidence boosted research in this area, searching for more potent and less toxic anticancer drugs. However, drug resistance and/or stability, high toxicity and low tumor selectivity are still major problems. Thus, there is constant urgency in the discovery of new drugs for cancer treatment. Present as secondary metabolites with high natural occurrence in edible plants and fruits, chromenes and coumarins have various and advantageous biological activities and many therapeutic applications. Due to their widespread existence, several natural chromenes and synthetic derivatives were already tested for anticancer properties both in vivo and in vitro. In this work, a number of new chromene-based compounds were prepared by simple and effective experimental procedures. The synthetic method involved the reaction of substituted salicylaldehydes and acetophenones, that led to α,β-unsaturated carbonyl compounds. These chalcones were then used as precursors of cyclic 4H-chromene derivatives, under appropriate and individually selected reaction conditions. An in vitro screening of the anticancer activity of the prepared α,β-unsaturated carbonyl compounds and chromenes was performed in a breast cancer model (MCF-7). Cell viability was evaluated for all compounds using the sulforhodamine assay and promising antitumor potential was found with IC50 values from 30 µM to 500 nM. For more consistent results, cellular viability will also be assessed using another breast cancer cell line (HS578t) and toxicity for normal cells will be evaluated using the MCF-10 model. The effect of these compounds will be further characterized, evaluating migration by the wound healing assay, invasion using matrigel invasion chambers, proliferation by BrdU incorporation and cell death by caspase-3 and annexin V/PI assays. New chromene-based compounds were synthesized by efficient synthetic methodologies and these derivatives demonstrated an interesting and promising anticancer profile.

No conflict of interest

F13. EMERGING ANTIBODY-BASED THERAPEUTIC STRATEGIES FOR BLADDER CANCER: A SYSTEMATIC REVIEW

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Introduction: Bladder cancer is the most common malignancy of the urinary tract but its management remains a clinically challenging problem. Despite antibody-based therapeutics have become established treatment options for over a decade in several types of cancer, bladder cancer has remained mostly an "orphan disease" regarding the introduction of these therapeutics. To shift this paradigm, several clinical studies involving antibodies targeting the most prominent bladder cancer-related biomolecular pathways and immunological mediators are ongoing. This work provides a systematic review on antibody-based therapeutic strategies for

bladder cancer currently undergoing clinical trial. Material & Methods: A comprehensive search of MEDLINE through PubMed and of Clinical Trials Registry was conducted with the query: ("Bladder Neoplasm"[All Fields] OR "Bladder Cancer"[All Fields] OR "Bladder Tumor"[All Fields] OR "Bladder Carcinoma"[All Fields]) AND ("Antibody"[All Fields] OR "Immunotherapy"[All Fields] OR "Antibody Therapy"[All Fields]). Procedures were conducted according to PRISMA guidelines. Results & Discussion: From an initial 2096 records, 29 clinical trials met full criteria for selection. The vast majority of the studies involving antibody-based therapeutics concern advanced stage bladder tumors (90%), which are associated with poor prognosis and lack effective therapeutics. Half of the approaches focused on targeting and inhibiting key molecules involved in oncogenic pathways, such as EGFR, HER2, VEGF, Ang, ALK1 and cell-adhesion, namely EpCAM. Other exciting emerging therapeutics focused on using antibodies for immune mediators aiming T cell cytotoxic activity stimulation, by blockage of CTLA-4, PD-1 and PD-L1. Such strategies have been proven capable of boosting highly specific immune responses against tumor cells and seem to be very promising as therapeutic agents, either alone or as combined strategies. Despite encouraging preclinical and clinical studies, only two phase III trials are currently being conducted targeting VEGF-A and both PD-L1 and PD-L2. Moreover, we note only modest improvements in patients' survival (bellow 12 months), irrespectively of the strategies, which will likely translate into few developments in bladder cancer management in near future. However, we believe that the lessons learned from ongoing clinical trials will surely allow the design of more effective strategies and lead to groundbreaking advancements in bladder cancer management.

No conflict of interest

F14. Generation and characterization of a fully human antibody against the interleukin-7 receptor, a potential target for T-ALL

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Introduction: Interleukin 7 (IL-7) is a cytokine produced in the bone marrow, thymus and other organs, required for normal T-cell development. However, IL-7 and the α subunit of its receptor (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 blasts. Moreover, 10% of T-ALL patients display IL-7Rα gain-of-function oncogenic mutations. These data suggest that the IL-7/IL-7Rα axis can be explored for the development of targeted therapies against T-ALL. Fully human monoclonal antibodies are particularly useful for pharmaceutical applications as they display reduced immunogenicity in humans. Using phage display technology we now describe the isolation and initial characterization of a fully human antibody against IL-7R. Materials and Methods: The phage display libraries ETH2 GOLD and PHILO DIAMOND were selected against a biotinylated recombinant extracellular domain of IL-7Rα. The isolated antibodies were produced in scFv format in bacterial cells, while the most promising clone was reformatted into human IgG1 and expressed in CHO cells. Both formats were characterized using size-exclusion chromatography, SDS-PAGE and Surface Plasmon Resonance experiments. Detection of IL-7Rα overexpressed in Ba/F3 cells was performed by flow cytometry. Results and Discussion: We generated a fully human monoclonal antibody specific to the human IL-7Rα using phage display technology. The antibody was expressed both as scFv fragment and as fully human IgG1. Purity and size of both formats were confirmed via size-exclusion chromatography and gel electrophoresis. BIAcore measurements revealed a 40.8(±17.5)nM dissociation constant for monomeric preparations of the scFv. Importantly, the antibody specifically recognizes the native antigen conformation in Ba/F3 cells expressing either wild type IL-7Rα, which heterodimerizes with CD132, or mutant IL-7Rα, which homodimerizes to elicit constitutive signaling. The fact that the antibody recognizes both IL-7R forms extends its potential clinical relevance for the treatment of IL-7R-expressing leukemias. We anticipate that the newly developed antibody may serve as building block for the development of novel therapeutic strategies, involving stimulation of anti-tumoral immunity and/or selective delivery of cytotoxic drugs or other bioactive payloads.

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:
Dario Neri is a cofounder and shareholder of Philochem AG

No conflict of interest

F15. Hyperbaric oxygen therapy sensitizes retinoblastoma cells to anti-cancer effects of photodynamic therapy

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Introduction: Retinoblastoma (RB) is the most common intraocular malignancy tumor in children with an incidence of 1 in 15000-20000 births. Photodynamic therapy (PDT) is an increasingly promising treatment for neuro-ophthalmologic cancer. PDT can induce cell death by increasing oxidative stress, through light activation of non-toxic photosensitizer (PS) molecules. Hyperbaric oxygen therapy (HBOT) consists in oxygen delivery to a patient, at pressures greater than atmospheric pressure. HBOT increases oxygen concentration in plasma and tissues, and has been shown to reduce inflammation and increase oxidative stress in animal models. Our aim is to evaluate the anti-cancer potential of the combination of PDT and HBOT in two different combination regimens, as an alternative approach for RB treatment. Materials and Methods: Human RB Y79 cells were cultured and submitted to different therapies: PDT alone, PDT+HBOT and HBOT+PDT. Cells were seeded in 96-multiwell plates, at a density of 0.5x106 cells/mL. Control cells were treated with PS solvent and increasing PS (ACS88F1) concentrations (5, 50, 100 and 500 nM). Moreover, HBOT was performed for 30 or 60 min, before (HBOT+PDT) and after (PDT+HBOT) light activation (10 J) of PS. Effects of combined therapies were assessed 24 h after treatments. Metabolic activity was evaluated by alamar blue assay and total protein content by SRB assay. Results and Discussion: Preliminary results showed that, after treatments, metabolic activity and protein content of RB cells decreased with increasing PS concentration and HBOT exposure time. With Alamar Blue assay, we verified that HBOT potentiates PDT treatment, being obtained a greater metabolic activity inhibition of Y79 cells compared to PDT alone. Preliminary results of metabolic activity evaluation also showed a better outcome for PDT+HBOT60min treatment. Moreover, results of SRB assay revealed a decrease of protein content with the combined therapies in comparison with PDT alone. In conclusion, we found that PS induced anti-proliferative effects. We observed a tendency for improved outcome with combined therapy, namely PDT+HBOT60min. Protein content evaluation revealed concomitant results. These studies may contribute to the development of a promising therapy for RB.

No conflict of interest

F16. Photodynamic therapy in combination with doxorubicin in osteosarcoma: Preliminary in vivo studies

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Introduction: Osteosarcoma (OS) is a malignant tumor that arises from primitive mesenchymal cells and is characterized pathologically by spindle cells and formation of osteoids. Doxorubicin (DOX) remains among the

most widely used antineoplasic agents in the treatment of a wide variety of solid tumors such as osteosarcoma. Combined treatments may target different key signal transduction pathways, may be more efficient in destroying cancer cells and in eluding cellular resistance mechanisms. Photodynamic therapy (PDT) is a non-mutagenic therapeutic modality for treating cancer. Several studies reported that combination treatment of chemotherapy and PDT may overcome tumor drug resistance, increase anticancer activity and became a therapeutic approach in cases that surgery is not possible. Animal models are good tools that we can use to allow understanding tumor biology and evaluating new therapeutic approaches. We aimed to evaluate the effect of the combination of photodynamic therapy and doxorubicin in an orthothopic animal model of osteosarcoma. Materials and Methods: MNNG-HOS (Human OS cell line) was propagated according to standard procedures. 20 Balb/c nu/nu nude female mice were injected in the skull with a suspension of 2×10^6 cells. Animals were divided on four groups. One control group (Group I) with 6 animals not submitted to PDT. Group II included 6 animals treated with a (5,15-bis(2-bromo-3-hydroxyfenil)porphyrin), a photosensitizer previously synthetized by our group, being administered intraperitoneally (2mg/kg) when tumor reached 200 mm3 of volume. Group III included 4 animals treated with a non-therapeutic dose of doxorubicin (2mg/kg). Group IV included 4 animals submitted to the combination of PDT and doxorubicin. After the injection of the photosensitizer (Group II and Group IV) the animals were protected from the light and after 72 hours the animals were irradiated with a Ceramoptec laser system. Animals were monitored daily and registered any signs of disease, during 12 days. Results and Discussion: In Group I we observed a solid round tumor that continues to grow along the time. The same happened with the animals of Group III. However the animals of the group treated with PDT (Group II) showed a decreased tumor volume when compared to the Group I and Group II. Regarding the preliminary results of the combination group (Group IV), volume of the tumors was the lowest of all groups. Follow up of the animals showed that the treatment were nontoxic and animals presented low variations of weight. This model allowed to conclude that PDT as a positive effect over the growth of OS tumor. The combination of PDT and doxorubicin seems to have a better outcome. These promising results will lead to further studies, in order to verify this approach as a new option for managing this cancer. Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER and Liga Portuguesa Contra o Cancro/ANA

No conflict of interest

F17. Synergistic effect of photodynamic therapy in combination with doxorubicin in osteosarcoma

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Introduction: Osteosarcoma (OS) is the most common malignant tumor that arises from primitive mesenchymal bone-forming cells. Doxorubicin (DOX) remains among the most widely used antineoplasic agents. Photodynamic therapy (PDT) is a non-mutagenic anticancer therapy. Several studies reported that combination therapy (CT) of chemotherapy and PDT may overcome tumor drug resistance, increase anticancer activity and become a therapeutic approach in cases where surgery is not possible. The main goal of this work is to evaluate the synergistic effect of the combination with PDT in OS cells. Materials and methods: MNNG-HOS human osteosarcoma cells were submitted to chemotherapy with DOX during 72 hours, or to PDT based in a photosensitizer (PS) (5,15-bis(2-bromo-3-hydroxyfenil)porphyrin), previously synthetized by plus light with a flux of 7,5 mW/cm2 or to the combination of both therapies. Metabolic activity and viability were evaluated through MTT and SRB assays. Furthermore, in order to evaluate the types of cell death, the mitochondrial membrane potential, cell cycle and oxidative stress (intracellular production of peroxides, superoxide anion and glutathione) we proceeded to flow cytometry technique. To evaluate the activity of superoxide dismutase (SOD) the WST-SOD Assay Kit (Sigma) was used. Results and discussion: The CT induced a significant decrease of metabolic activity and of cell viability to values of (16,48±5,35)% (p<0,001) and of (46,60±20,35)% (p=0,001),

respectively. Furthermore, analysis of the metabolic activity results obtained showed a combination index of 0,541, which suggests a synergistic effect. Regarding flow cytometry studies there was a sharper loss of mitochondrial membrane potential in cell cultures subjected to CT to 3,61±2,86 (p=0,006) and the type of predominant cell death induced by therapy was later apoptosis/necrosis. In terms of cell cycle, there was a retention in G2/M phase in cells subjected to chemotherapy (31,00±9,24)% (p=0,002) and cells subjected to CT (29,38±12,16)% (p=0,006), which is an indication of cell death by apoptosis. The cells subjected to CT also showed a higher intracellular production of reactive oxygen species, namely peroxides, that increased 2,19 ± 0,37% (p < 0,001) relative to control, and superoxide anion, that increased 4,09±1,17 (p<0,001) relative to control. Regarding antioxidants defenses there was a decrease in glutathione level while preliminary SOD activity results showed an increase in cells subjected to CT 3,42±3,58. The CT presented a synergistic cytotoxic effect on OS cells. These results reveal the potential of this combination as a future therapeutic approach in OS. Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER and Liga Portuguesa Contra o Cancro/ANA.

No conflict of interest

F18. The mechanism of cell death by photodynamic therapy combined with acetylsalicylic acid in colon and esophagus cancer cells

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Introduction: The colorectal and esophageal cancers are some of the most common cancers in terms of incidence and mortality in both man and women, having both of them a high cyclooxygenase expression. Photodynamic therapy is a low invasive therapy that is promising for the treatment of various disorders, particularly cancer. The objective of this study was to evaluate the use of cyclooxygenase inhibitors such as acetylsalicylic acid (aspirin) in combination with photodynamic therapy as a synergistic therapy promoting anti-proliferative effects in WiDr and OE19 cancer cells. Materials and Methods: WiDr cell line was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% antibiotics and sodium piruvate, while OE19 cell line was propagated in culture with RPMI also with 10% FBS, 1% antibiotics and sodium piruvate. For the studies of cytotoxicity evaluation, 80.000 cells/ml (MTT assay) or 250.000 cells/ml (SRB assay) were plated in 48 or 24 multiwell plates, with the addition of a photosensitizer, previously synthetized by us, at the concentrations of 5 nM, 50 nM, 200nM and 500 nM past 24h. Subsequently the plates were irradiated with a flow 7,5mW/cm2 to achieve 10J, followed by the addition of aspirin at the concentrations of 2,5mM and 10mM. After 24 hours cell metabolic activity and cell viability was assessed by the MTT assay and SRB assay, respectively. For the flow cytometry studies, the cell cultures were stained with annexin V/propidium iodide-FITC probe for evaluating cell death type and propidium iodide for evaluating cell cycle. Results and Discussion: The results following the MTT Assay and SRB Assay suggest that the combination of photodynamic therapy with acetylsalicylic acid diminishes both cancer cell lines metabolic activity and viability in a manner dependent of the concentrations of the photosensitizer, with a decrease of 82±10% in WiDr cell line and 79±10% in OE19 cell line in terms of metabolic activity for the treatment with PS 50nM+2,5mM Aspirin. Considering flow cytometry, cell death by apoptosis and necrosis occurs in both cell lines, in a manner dependent of photosensitizer concentration. In terms of cell cycle, there is a measurable retention in Early G0 phase in some conditions, which is an indication of cell death by apoptosis. The combination of photodynamic therapy and acetylsalicylic acid is potentially synergistic being dependent on the concentration of the photosensitizer in both cell lines, playing also role in the type of cell death activated in both cell lines studied. Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER

No conflict of interest

F19. Acetysalicylic acid as a possible enhancer of reactive oxigen species and cell death in photodynamic therapy

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Introduction: One of the key features for the overall efficacy of photodynamic therapy is the generation of reactive oxygen species (ROS), which are the main effectors in terms of cell death. Non-steroidal anti-inflammatory drugs, like acetylsalicylic acid are referred often in studies as promoters of ROS generation although others paradoxically refer its properties in terms of cytoprotection by antioxidative mechanisms. Having this in mind, the objective of this study is to understand if acetylsalicylic acid interacts with photodynamic therapy enhancing the ROS generation in WiDr and OE19 cell lines leading to cell death. Materials and Methods: WiDr cell line was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% antibiotics and sodium piruvate, while OE19 cell line was propagated in culture with RPMI also with 10% FBS, 1% antibiotics and sodium piruvate. For the studies of ROS inhibition 80.000 cells/mL were seeded in 48 well plated, with the addition of a photosensitizer, previously synthetized by us, in concentration range from 5nM to 500nM. After 24h of incubation, 5mM Sodium Azide (Superoxide anion inhibition) or 40mM D-Mannitol (Hydroxyl radical inhibition) were added to the plates. Past 2h the plates were irradiated with a flow 7,5mW/cm2 to achieve 10J, followed by the addition of aspirin at the concentration of 2,5mM. The metabolic activity was measured by MTT Assay 24h later. For the flow cytometry studies, the cells were stained with DCF probe for evaluating the peroxide content, DHE probe for evaluating the superoxide anion formation and JC-1 probe for evaluating the mitochondrial membrane potential. Results and Discussion: Following the inhibition of superoxide anion and hydroxyl radical the efficacy of photodynamic therapy is limited. The intracellular peroxide content seems to decrease with higher photosensitizer concentrations particularly in WiDr cell line, decreasing almost 4-fold in the higher concentration. In terms of superoxide anion, the higher photosensitizer concentration leads to an increase of its intracellular concentration. Finally, is observed a decrease in mitochondrial membrane potential in both cell lines, with a 2-fold decrease for the WiDr cell line and a 3-fold decrease for OE19 cell line, which is a marker of cell death by apoptosis. Reactive oxygen species are of paramount importance in photodynamic therapy, being singlet oxygen, typical of type II photodynamic reaction, considered the most prevalent one. However with this work we reinforce the importance of type II ROS in anticancer activity. Furthermore, acetylsalicylic acid may have a role in modulating the ROS generation after photodynamic therapy. Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER

No conflict of interest

F20. Photodynamic therapy with a novel photoimmunoconjugate improves currently available anti-HER2 gastric cancer therapy efficiency

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Introduction: Gastric cancer (GC) is the 3rd leading cause of cancer mortality worldwide. Most newly diagnosed patients present with advanced and unresectable disease for which chemotherapy is the main treatment option. Anti-HER2 therapy with monoclonal antibody trastuzumab, has been added to conventional therapy for advanced and unresectable diseased patients. Nevertheless, trastuzumab-based therapy only improves overall survival (OS) in ~3 months, when compared to chemotherapy alone (OS<1 year). There is strong need to improve anti-HER2 therapeutic approaches to improve OS in ~20% of HER2 positive cases. Here, we propose that photodynamic therapy (PDT) with a novel porphyrin modified trastuzumab can improve the currently available anti-HER2 targeted therapy against GC. Material and Methods: Trastuzumab was conjugated with a cationized porphyrin by the N-hydroxysuccinimide strategy. Trast:porph conjugation was confirmed by MALDI-TOF/TOF analysis and immunoreactivity by ELISA and flow cytometry; its cellular localization was assessed by immunofluorescence; and PDT effects on cell growth/viability by resazurin-based and live/dead assays. HER2-positive cancer cells were subcutaneously inoculated in nude mice, randomized into 3 groups for PDT: 1) vehicle; 2) trastuzumab; 3) trast:porph. Two cycles of PDT were performed and tumor growth kinetics and proliferation assessed. Results and Discussion: Trast:porph maintained immunoreactivity and improved cell internalization and co-localization with the lysosomal marker LAMP1, when compared with native trastuzumab. Trast:porph induced cell death solely in HER2 positive GC cells, which was neither observed for unconjugated antibody nor for non-armed porphyrin. In vivo studies showed that trast:porph delayed tumour growth in comparison to trastuzumab alone. Tumour re-growth was observed 6 days after ending PDT, supporting its growth restrictive effect and the need for additional PDT cycles. Our results show that PDT with the novel Trast:porph improves currently available anti-HER2 therapy efficiency in GC. Support: FEDER/COMPETE/ FCT projects: PEst-C/SAU/LA0003/2013; FCT/MEC/PT2020 nº007274-UID/BIM/04293); ON.2/QREN projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067; Marie Curie ITN grant no. 316975/2012 and Ph.D. grant to BK; FCT Fellowships (PD/BI/113971/2015 to SR and SFRH/BD/113031/2015 to CP;) and Salary support to GMA from the iFCT Program 2013 (IF/00615/2013), POPH - QREN Type 4.2, ESF and MCTES.

No conflict of interest

F21. Role of PI3K and autophagic inhibitors on the sensitization of resistant ER+ breast cancer cells to Exemestane treatment

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Introduction: Aromatase inhibitors (AIs) are one of the therapeutic approaches for estrogen-receptor positive (ER+) breast cancer, being Exemestane (Exe) the third-generation steroidal AI used in clinic. Besides its therapeutic success, acquired resistance may develop causing tumor relapse. Thus, it is important to search for new strategies to surpass Exe-acquired resistance. Our group showed that Exe induces apoptosis and autophagy in sensitive breast cancer cells that overexpress aromatase (MCF-7aro), being autophagy a pro-survival process that may be involved in resistance [1, 2]. Besides PI3K/Akt is considered the major pathway in endocrine resistance. Therefore, using an AI-resistant breast cancer cell line (LTEDaro) and a sensitive MCF-7aro cell line it will be investigated if autophagy and PI3K/Akt inhibition play roles on the Exe-resistance process. Matherial and Methods: The effects on Exe-treated LTEDaro/MCF-7aro cells exposed to PI3K inhibitors (Wortmannin, (WT), Ly294002 (LY)) and to the autophagic inhibitor, Spautin-1 (SP) were studied by MTT and LDH assays. Akt and p44/42 phosphorylations and PI3K expression were evaluated by Western-blot analysis. Autophagy involvement was evaluated by Acridine Orange staining and the occurence of apoptosis was confirmed by caspases-7 and -9 luminescence assays. Results and Discussion: LY induces a reduction in viability of Exe-treated LTEDaro cells, while it caused no effects in the Exe-treated MCF-7aro cells. Moreover, results suggests that in Exe-treated LTEDaro cells, LY does not inhibit autophagy while it inhibits PI3K/Akt pathway activation. WT does not affect Exe-treated MCF-7aro cells, though, it decreases viability of Exe-treated LTEDaro cells. SP seems to have a protective role in the Exe-treated MCF7aro cells, but on contrary it decreases Exe-treated LTEDaro cell viability. Besides the anti-proliferative effects, all the compounds, except SP, appears to

increase caspase-9 and -7 activities in the AIs-resistant breast cancer cells. Thus, by modulating PI3K/Akt pathway and autophagy it may be possible to sensitize acquired-resistant breast cancer cells to Exe therapy, by inducing apoptosis. This work provides new insights in breast cancer by contributing to the elucidation of the mechanisms and targets involved in Exe-acquired resistance.

No conflict of interest

F22. Development of AAV-shRNA vectors for human basal-like breast cancer therapy

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Introduction Basal-like breast cancer (BBC) is mainly comprised by triple-negative breast cancers (TNBC) which display a highly aggressive and metastatic phenotype. These tumors rapidly acquire resistance to chemotherapy, are refractory to endocrine therapy and have no targeted therapeutic options. Consequently, they present a poor clinical outcome. Therefore, there is a need to find alternative therapies. Here we aimed to develop a novel treatment for BBC based on viral vector-mediated delivery of shRNA targeting BBC cell survival genes. Materials and methods pAAV2-shRNA plasmids were generated by cloning shRNA sequences targeting BBC dependency genes together with a GFP reporter between AAV2 inverted terminal repeats in an AAV plasmid backbone. AAV2-shRNA vectors were produced by 2-plasmid transfection of HEK293T cells with pAAV2-shRNA and pDG plasmids and purified by affinity chromatography. Target gene knockdown and functional effects of the different AAV2-shRNA vectors in BBC cells were evaluated by qRT-PCR, Presto Blue analysis and flow cytometry. The anti-tumorigenic effect of AAV2-PSMA2sh vector was evaluated on mouse orthotopic BBC cell xenografts. Results and discussion AAV2-shRNA vectors against several BBC genes and control scramble vectors were produced with high titers and purity. Analysis of the target gene knockdown of the different AAV2-shRNA vectors in BBC MDA-MB-468 and HCC1954 cell lines showed that transduction with AAV2-PSMA2sh decreased the expression of its target gene by 80%, as compared to control cells transduced with AAV2 vector encoding a scramble shRNA (negative control). In MDA-MB-468 cells, the decreased expression levels of PSMA2 gene were associated with significant decrease of cell viability and 2 fold increase in the percentage of apoptotic cells. In vivo studies to assess the anti-tumorigenic effect of AAV2-PSMA2sh in mouse orthotopic BBC cell xenografts showed that intratumoral injections with this vector caused a significant decrease in tumor growth when compared to negative scramble shRNA control vector or PBS injected mice. No major macroscopic alterations were observed in the liver of mice treated with AAV-shRNA vectors. These results, though preliminary, indicate that the AAV2-PSMA2sh vector developed herein has the ability to decrease BBC tumorigenesis. Further assays are currently in progress to validate these findings, to quantify PSMA2 silencing and to characterize the proliferation and apoptotic status of tumors.

No conflict of interest

F23. Can Cold Atmoshperic Plasma selectively ablate Retinoblastoma cells?

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Background: Retinoblastoma is a malignant tumor arising from the nuclear layer of the retina, being the most common primary intraocular tumor in children. Histologically, areas of necrosis found in relatively avascular areas demonstrate the dependence of retinoblastoma on its blood supply. Currently available therapy methods lack selectivity and are associated with serious side effects as loss of vision and secondary neoplasias. Plasma, the fourth state of matter, is a gas with enough energy to ionize a significant fraction of atoms or molecules, forming positive ions, electrons and reactive species. The aim of this work was to evaluate the effect and selectivity of cold atmospheric plasma (CAP) in human retinoblastoma. Materials and Methods: We developed an electronic device capable of generating high output voltage (HV;~4kV). This equipment was designed to initiate an electrical discharge between the HV electrode and multiwell plates where cell cultures acted both as the grounded electrode and the target. Human retinoblastoma Y79 cells were seeded in the multiwell plates, in volumes of 200μl at a concentration of 5×10^5 cells/ml and left overnight. For treatment, CAP was generated in open air, 2 mm above the surface of the cell cultures medium. Several short periods of time, ranging from 15s to 180s, were tested. In order to evaluate cytotoxicity, Alamar Blue and MTT assays were performed. In addition, cell viability was assessed by SRB assay. Concerning selectivity, human fibroblasts HFF1 were treated similarly and Alamar Blue was performed. Furthermore, in order to evaluate the potential anti-angiogenic effects of CAP, an aortic ring assay was carried out in excised mouse thoracic aorta segments embedded in collagen matrix and cultured in 96 multiwell plates. All the tests were performed 24 hours after CAP exposure. Results and Discussion: After CAP treatment, the metabolic activity of human retinoblastoma cells significantly decreased accordingly to the exposure time. After 60s of CAP exposure, the observed metabolic activity was $(37.5 \pm 19.7)\%$ ($p < 0.001$) and $(57.2 \pm 21.2)\%$ ($p = 0.047$) as evaluated by Alamar Blue and MTT assay, respectively. Furthermore, the time calculated to reduce this activity in 50% (IT50) was 68.1s and 79.2s, accordingly to the previous techniques. Moreover, the protein content of $(56.3 \pm 19.6)\%$ ($p = 0.048$) was obtained after the same time exposure as determined by SRB assay, revealing cell death as the cause of these results. However, regarding the fibroblasts, after 60s of CAP exposure the metabolic activity was still $(74.5 \pm 13.1)\%$ ($p = 0.058$) and the calculated IT50 was 109.6s. These results suggest that CAP might be selective to tumor cells ($p < 0,001$). Furthermore, our preliminary results on aortic ring assay revealed that CAP might have anti-angiogenic effects after only 60s, therefore, it might have a possible deleterious effect on these vessel dependent tumor cells. Funding: FCT, Portugal (UID/NEU/04539/2013), COMPETE-FEDER

No conflict of interest

F24. Exploring the effect of multifunctional polymer-ruthenium conjugates: new promising anticancer agents

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A new family of multifunctional polymer-ruthenium conjugates for targeted delivery of chemotherapeutic agents has been synthesized and tested as anticancer agents. These drugs might provide potential tools to surmount many of the current limitations in conventional chemotherapy, including undesirable biodistribution, drug resistance and severe systemic side effects. Our approach constitutes an innovation relatively to other high molecular weight drugs reported in the literature mainly because an exact control of the amount of our cytotoxic drug in the polymeric chain is achievable due to the fine tune functionalization of the polymers (other approaches use a variable percentage of metal per quantity of drug). A degradation study suggested a pH-dependency which is important considering drug delivery, since pH of most solid tumors range from pH 5.7 to pH 7.2 while in normal blood remains well-buffered and constant at pH 7.4. The effect of these compounds on cell proliferation was evaluated by sulforhodamine B assay in order to determine the IC50 values in the breast cancer cell line, MDA-MB-231 and in the colorectal cancer cell lines, SW480 and RKO. Our results show that these conjugates efficiently inhibit MDA-MB-231 proliferation at concentrations lower than those needed to exert the same effect with cisplatin, a chemotherapeutic agent used in breast cancer therapy. Additionally,

these compounds also inhibited cells proliferation in SW480 and RKO cells at low concentrations. Further studies are needed in order to better understand the effects of these compounds and identify their cellular targets, although our preliminary results suggest that these compounds are potent compounds and might be used as wide-ranging anticancer agents. Acknowledgements The authors thanks FCT, The Portuguese Foundation for Science and Technology, within the scope of the project PEst-OE/QUI/UI0536. Andreia Valente acknowledge the COST action CM1302 (SIPs) and the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE - European Social Fund). This work was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through the COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI).

No conflict of interest

F25. Cathepsin D-mediated degradation of damaged mitochondria rescues colorectal cancer cells from acetate-induced apoptosis.

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Acetate is a short-chain fatty acid secreted by Propionibacteria from the human intestine, known to induce mitochondrial apoptotic death in colorectal cancer (CRC) cells. We previously established that acetate also induces lysosome membrane permeabilization in CRC cells, associated with release of the lysosomal protease cathepsin D (CatD), which has a well-established role in the mitochondrial apoptotic cascade. These results mimicked our previous data in the yeast system. Unexpectedly, we showed that CatD has an antiapoptotic role in this process, as pepstatin A (a CatD inhibitor) increased acetate-induced apoptosis. Here, we aimed to assess the role of CatD in acetate-induced mitochondrial alterations. We found that inhibition of CatD with siRNA or pepstatin A, enhances apoptosis associated with higher mitochondrial dysfunction such as an increase in ROS accumulation (total and mitochondrial), and also with increase in mitochondrial mass and mitochondrial potential depolarization. This demonstrates that the role of CatD in mitochondrial degradation depends on its protease activity during acetate-induced apoptosis in colorectal cancer cells. We further show that CatD, but not CatB or CatL, is involved in the degradation of the dysfunctional mitochondria to enhance CRC cell survival in response to acetate, associated with impaired autophagy. Our data therefore indicates that acetate-induced apoptosis involves a cross-talk between the lysosome and mitochondria, and support sensitizing CRC cells to acetate-induced apoptosis by inhibiting CatD as a novel therapeutic strategy in CRC. Acknowledgments This work was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through the COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by Fundação para a Ciência e Tecnologia through projects PEst-OE/BIA/UI4050/2014 and FCTANR/BEX-BCM/0175/2012, as well as fellowships to C.S.F.O. (SFRH/BD/77449/2011), H.P. (SFRH/BD/73139/2010), L.C. (SFRH/BD/93589/2013) and S.C. (SFRH/BPD/89980/2012). This work was originally published in: Cell Death Dis. 2015 Jun 18;6:e1788. doi: 10.1038/cddis.2015.157.

No conflict of interest

G1. Hybrid lipid-polymeric nanoparticles as cancer immunotherapeutics

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Introduction: Hybrid lipid-polymeric nanoparticles (HL-NPs) stand out as potential drug delivery candidates. This study aimed to design a therapeutic HL-NPs cancer vaccine able to deliver entrapped antigens and immune modulators to dendritic cells (DCs) for eradication of primary/metastatic cells by strengthening the host immune response. Materials and Methods: HL-NPs were prepared by the double emulsion-solvent evaporation method. Two different lipids, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (POPC) and 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol (DMPG), were used to modify the Poly(lactic-co-glycolic acid) (PLGA) matrix. HL-NPs hydrodynamic diameter and polydispersity index were determined by Dynamic Light Scattering; surface morphology was evaluated by Atomic Force Microscopy (AFM) and zeta potential (ZP) was determined by Laser Doppler Electrophoresis. The entrapment efficiency (EE) and loading capacity (LC) were quantified by fluorescence using OVA Alexa Fluor® 647 conjugate as a model antigen. The viability of DCs in the presence of the HL-NPs was inferred using AlamarBlue® Assay, while the internalization of the HL-NPs by these phagocytic cells was evaluated by flow cytometry. Results and Discussion: The mean diameter of HL-NPs (137 nm) was lower than the one presented by the polymeric ones (199 nm). All formulations presented a monodispersed population, ZP close to neutrality and high EE and LC values (EE ≥ 69.91 % (w/v), LC≥ 3.52 µg/mg). AFM analysis evidenced that the addition of lipids to the PLGA matrix resulted in smoother nanoparticle surfaces. Nanoparticle treated cell viability was close to 100%, and nanoparticle internalization levels by DCs increased with the incubation time and absence of lipids. However, it is expected that the higher EE and LC observed for HL-NPs will overcome those lower levels of internalization. In conclusion, a promising hybrid nanoplatform for antigen delivery and DC activation and maturation was developed. In vivo studies, in a metastatic malignant melanoma mouse model, are ongoing to evaluate if the HL-NPs are able to induce a selective and extensive immune response capable to elicit reduction of tumor growth or even its eradication. The authors are grateful to: i) Fundação para a Ciência e a Tecnologia, Ministério da Ciência e da Tecnologia Portugal for iMed.ULisboa grant UID/DTP/04138/2013, UTAP/ICDT/DTPFTO/0016/2014 research project and PhD grant SFRH/BD/87869/2012; and ii) EPSRC (Engineering & Physical Sciences Research Council) Centre for Innovative Manufacturing in Emergent Macromolecular Therapies.

No conflict of interest

G2. Microglia foster glioma cells to co-opt stem-like features for tumor aggressiveness

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Introduction: Glioblastoma multiform (GBM) is a highly lethal brain tumor containing self-renewing glioma stem-like cells (GSC) that sustain tumor growth, invasiveness and therapeutic resistance. Microglia (MG), the resident brain macrophages are highly abundant in GBM and adopt an activation phenotype that promotes tumor growth and invasiveness, instead of halt disease progression. Recent studies point to a direct interaction of GSC with MG cells suggesting they represent an important niche component that modulates the acquisition/maintenance of a stem-like phenotype. We investigated whether the bi-directional interactions between glioma cells and MG modulate the malignant behavior of GBM and how it contributes to the induction of a stem-like phenotype. Material and methods: A MG cell line (BV-2) was exposed to glioma-conditioned medium (CM) or co-cultured with human GBM cells lines (U87 and U118). The activation status of MG was assessed by measuring the cellular uptake of an inflammatory marker 11C-PK11195, release of nitric oxide (NO) and expression of iNOS by qRT-PCR. Transcriptional analysis of anti-and pro-inflammatory cytokines was determined by qRT-PCR. In parallel we evaluated the reciprocal effects of MG on glioma cells proliferation, chemoresistance, invasiveness and stemness properties, based on the sphere-forming efficiency and ALDH activity. Results and discussion: We observed that GBM-derived soluble factors polarized MG towards a distinct inflammatory activation phenotype characterized by enhanced NO production and iNOS expression and increased uptake of 11C-PK11195. Transcriptional analysis of MG revealed an intermediate activation status distinct from the classical M1 phenotype or alternative M2 activation state, characterized by an up-regulation of TNF-α, IL-6, IL-10 and ARG-1. Co-culture with MG, increased the proliferation rate and migration of GBM cells and their chemoresistance to temozolomide. Furthermore, we observed an increased expression in the stem cell markers CD133 and Nestin as well as on ALDH activity and sphere-forming efficiency in GBM cells. These results suggest that MG, under the influence of GBM cells, creates a permissive inflammatory microenvironment that exacerbates the malignant behavior of GBM cells, in part by co-opting for a stem-like phenotype, considered a major obstacle to successful cancer treatment. Funding: FCT (Strategic Project PEst-C/SAU/UI3282/2011-2013 and UID/NEU/04539/2013) and COMPETE-FEDER.

No conflict of interest

G3. In vitro reconstruction of the tumor microenvironment: integrating immune-competent cells

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Introduction: Tumor development, progression and treatment are strongly influenced by its complex microenvironment, in which the immune-competent cells play a critical role. Indeed, most aggressive solid tumors are often associated with macrophages which are described to have a tumor promoting function – Tumor associated macrophages (TAMs) 1. Nevertheless, most in vitro tumor models do not allow the study of the tumor-immune cell interactions. Materials and methods: The strategy herein presented is based on aggregation of tumor cells in stirred-tank culture systems and microencapsulation to enclose tumor cell aggregates with fibroblasts and immune competent cells in alginate capsules. The non-small cell lung carcinoma (NSCLC) cell line NCI-H157 was co-cultured with human immortalized cancer associated fibroblasts (CAFs) and human monocytic cells (THP1 cell line and blood-derived monocytes). The triple co-cultures were maintained in parallel with tumor mono- and double co-cultures (tumor-CAFs and tumor-monocytes) for long-term in stirred-tank systems and the tumor phenotype and tumor-immune cell crosstalk was assessed. Results and discussion: Microencapsulation of NSCLC cell aggregates with fibroblasts and monocytes resulted in viable cultures over 3 weeks. Tumor cell concentration increased throughout the culture time both in mono-, double- and triple co-cultures. Mesenchymal markers such as N-cadherin, vimentin and nuclear β-catenin were detected in tumor cell aggregates of triple co-cultures. Moreover, histological characterization revealed differences in tumor cell morphology and aggregate area, which was reduced in triple co-cultures but with a higher number of cell aggregates. Cytokine profiling revealed qualitative and quantitative differences betwen the different culture types, with identification of potential tumor-immune cell crosstalk mediators. Ultimately,

the developed cell model was applied in drug screening, to evaluate efficacy of standard and immune-mediated therapies. In summary, herein is presented a novel cell model that integrates the immune cell compartment in interaction with tumor cells and fibroblasts. Importantly, this system allows long-term monitoring, being suitable to follow the effect of immune component on tumor progression. This constitutes a new tool for the study of cancer immunobiology and for pre-clinical assessment of immune-mediated innovative treatments. References: 1. Fukuda K. The Role of tumor-associated macrophage in tumor progression. Front Biosci. 2012;S4:787. doi:10.2741/S299. Acknowledgments: This research received support from the Innovative Medicines Initiative Joint Undertaking (grant agreement n° 115188), FCT (iNOVA4Health—UID/Multi/04462/2013). MFE and SA are recipients of PhD fellowships from FCT (SFRH/BD/52208/2013 and PD/BD/105768/2014).

No conflict of interest

G4. An interferon-γ-delivery system based on chitosan/poly-γ-glutamic acid nanoparticles as cancer immunotherapy

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Introduction: The modulation of immune response has been used as a strategy to enhance the efficacy of anti-cancer therapies. However, most of the immunotherapeutic approaches have limited success due to the immunomodulation mediated by the tumour microenvironment. Macrophages can be used as promising targets of novel anti-cancer therapies due to their functional plasticity, being able to adapt to the microenvironment led to the identification of two major differentiation phenotypes: i) the M1-like macrophages, characterized by the pro-inflammatory profile and ii) the M2-like macrophages, characterized by their anti-inflammatory capacity that dampen the immune response and consequently, favour tumour progression. These cells have been reported to affect angiogenesis, tissue remodelling, cancer cell motility and invasion, promoting tumour progression. Recently, we described the M2-like macrophages are the most efficient stimulators of gastric and colorectal cancer cell invasion, motility/migration and proteolysis. Our current aim is to develop a new therapy to modulate macrophage differentiation towards an anti-tumour phenotype, preventing cancer cell invasion and avoiding metastasis. Recently, we reported a drug delivery system, based on the incorporation of interferon (IFN)-γ, a cytokine with immunostimulatory/anti-tumour properties, in chitosan (Ch)/poly-γ-glutamic acid (γ-PGA) multilayers. These complexes had the ability to modulate in vitro the macrophage phenotype, converting them from a pro-tumour M2-like into an anti-tumour M1-like phenotype. Material and Methods: For a therapeutic strategy, we are currently developing a delivery system, based on these IFN-γ-Ch/Poly-γ-PGA polyelectrolyte complexes by co-acervation method. Results and Discussion: With this method, nanoparticles with 182 ± 5.2 nm and 0.26 ± 0.01 polydispersion index were formed. These nanoparticles seem to revert IL-10-stimulated macrophages (M2-like) into an M1-like phenotype, inhibiting macrophage-mediated colorectal cancer cell invasion. Overall, these results suggest a novel therapeutic strategy to allow simultaneous immunomodulation of the tumour microenvironment while reversing macrophage-mediated cancer cell invasion.

No conflict of interest

G5. Human colorectal tumour decellularized matrices polarize macrophages towards a pro-invasive phenotype

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Introduction: Tumours are complex microenvironments where, besides cancer cells, there are other stromal cells and an extracellular matrix (ECM). The fact that a cancer is more than just tumour cells is being perceived as a great opportunity for cancer prevention and treatment. In this context, macrophages emerged as modulators of cancer progression, regulating breast cancer cell migration, invasion and metastasis. In a simplistic vision of macrophage function in tumours, these have been described as key elements for carcinogenesis, preventing the establishment and spreading of cancer cells – M1 macrophages – or supporting tumour growth and progression – M2 macrophages. We are particularly interested in elucidating how ECM modulates macrophage polarization. Therefore, we developed an innovative 3D-organotypic culture mode, by decellularizing human colorectal cancer tissue fragments and by repopulating them with monocytes, mimicking more closely the natural tumour microenvironment. Materials and Methods: We optimized the decellularization protocol and accessed its efficiency. Matrices were characterized by immunohistochemistry (IHC) and Scanning Electron Microscopy. These matrices were repopulated with freshly isolated primary human monocytes which were allowed to differentiate for 14 days. Macrophage differentiation was analyzed by IHC, ELISA, zymography and real-time PCR. Finally, we evaluated the impact of these macrophages on cancer cell invasion, in vitro. Results and discussion: DNA quantification and DAPI staining confirmed the efficiency of the decellularization method. SEM analysis revealed an ECM fiber meshwork with a very similar architecture to the native tissues. Decellularization reduced GAGS content but other ECM components were retained. Repopulation experiments clearly evidenced that monocytes were able to colonize these decellularized matrices and to differentiate into macrophages. Moreover, normal and tumour-derived matrices distinctly modulated macrophage differentiation. Our results consistently revealed that macrophages repopulating normal decellularized matrices present higher MMP-9 activity and secrete higher levels of IL-6 but lower levels of IL-10. Real-time PCR analysis revealed that monocytes differentiated on tumour decellularized matrices express less CCR7, TNF and MMP1 but significantly more CCL18. Interestingly, macrophages repopulating tumour matrices were more efficient in stimulating RKO cancer cell invasion than those repopulating normal matrices. Conclusions: These results clearly highlight that differences between normal and tumour matrices have a critical impact on macrophage polarization and reinforce the relevance of using in vitro models that resemble the native microenvironments. Importantly, it opens new perspectives for the design of novel therapeutic strategies that also target the ECM.

No conflict of interest

H1. Hypofractionation Strategies Assessment for Early Breast Cancer

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Keywords: Radiosensitivity, hypofractionation, early breast cancer, cardiovascular disease Introduction In Portugal it is estimated that one in every 11 women will develop breast cancer. Radiation therapy is commonly given to breast cancer patients, often after surgery, in order to minimize the risk of local recurrence in the conserved breast, chest wall, and lymph nodes. The combination of surgery and subsequent radiation therapy reduces the risks of local recurrence by 50% and long-term breast cancer mortality by 17%. In modern radiotherapy treatment planning there are 3 main areas to be explored in the field of dosimetry: Margins, static or dynamic fields and fractionation. Fractionation in radiotherapy was initiated in order to spare normal tissue through repair of sublethal damage and repopulation of surviving cells, as well as increase the damage caused in the tumour by reoxygenation of hypoxic cells and redistribution of cells through the cell cycle. Radiosensitivity gives us the relative susceptibility of cells, where different tissues have different behaviours during treatment. Radiosensitivity assess tumour response to treatment and this is reflected in the α/β ratio of the linear quadratic model (LQM). This is an important point of difference when comparing the responses of normal tissues, responsible for the most important adverse late effects, which are sensitive to the size of the fraction and the total dose. Hypofractionated radiation therapy, using fraction sizes larger than 2.0 Gy and reduced total treatment time has become an established alternative to the standard fraction, showing good ratios of the local tumour control and overall survival of women in long follow-up periods. This work, intendeds to firstly assess the fractionation strategy with regard to the reduction of the overall treatment time and the increase in dose per fraction to the boost volume, exploiting the radiobiological differences in acute and late response tissues through the LQM. We also intend to compare the equivalence of conventional and hypofractionated fractionation schemes analysing the possible advantages and/or disadvantages of dose distributions and fractionation. Materials and Methods The evaluation of fractionation strategies was performed using Matlab version 8.1.0.604 R2013a in order to exploit the radiobiological differences, through the linear quadratic model (LQM). Several parameters were studied, such as the tumour response to radiation, α/β ratio, fractionation schedules used in radiotherapy treatments. To assess the equivalence of the fraction schemes (conventional or hypofractionation) and the possible advantages or disadvantages of dose distributions, treatment plans of ten patients with left breast cancer were simulated, in order to compare both techniques. Treatment plans were generated using the treatment planning system (Eclipse® version 13.5). The dosimetric comparison focused on the whole breast irradiation phase and the additional dose to the tumour bed was not considered. The results were accomplished using the IBM SPSS Statistics version 21.0.0.0 for comparison the average doses between schedules. The Wicoxon-Mann-Whitney test was used because it is a non-parametric test used for two independent samples. Results and Discussion For breast cancer, local tumour control and delayed response of normal tissues have a correlation with the dose per fraction, when, α/β is equal to 3 Gy. Radiotherapy schedules used were: 50 Gy in 25 fractions, for the conventional schedule and 40 Gy in 15 fractions or 42.5 Gy in 16 fractions for hypofractionation. The average dose administered to the Clinical Target Volume (CTV) in the conventional schedule was of 51,03 Gy with a standard deviation (SD) of 0.46, while in hypofractionation the average dose was of 40.06 Gy with a SD of 0 19. From our results it was possible to determine that the coverage of the target volume was more precise and with less hot spots in the hypofractionated schedule. The average doses to the heart for patients whose left breast was irradiated, were 7.43 Gy and 5.82 Gy for conventional or HF fractionation, respectively. This is quite relevant considering that cardiovascular disease (CVD) is predominant cause of mortality in breast cancer survivors over 60 years of age, with the average dose to the heart being the best predictor of radiation induced CVD. The question arises whether hypofractionated radiotherapy should be used as the preferred schedule for adjuvant radiotherapy in all breast cancer patients. Patients below 40 years, with locally advanced breast cancer, and those having

undergone mastectomy were not well represented in the hypofractionation trials. Consequently, some national treatment guidelines do not recommend hypofractionated radiotherapy for these patients, whereas others do. From a radiobiological point of view, it is unlikely that hypofractionated radiotherapy in younger patients or patients after mastectomy or those with locally advanced cancer would result in substantially different outcomes.

No conflict of interest

H2. Histone H2AX phosphorylation after ionizing radiation in a human-derived renal proximal tubular cell line (HKC-8)

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Introduction: Kidneys are major dose-limiting structures in abdominal external beam radiotherapy, mainly used in the treatment of gastrointestinal malignancies. Because kidneys are inherently radiosensitive, the correspondent dose tolerance limits are lower than other surrounding organs in abdomen. Ionizing radiation (IR) induces a wide variety of DNA lesions including double-strand breaks (DSBs). DSBs are considered to be the main cause for cell death and, if unrepaired or improperly repaired, can lead to chromosomal aberrations and ultimately to pathological disorders such as cancer. Materials and Methods: H2AX is phosphorylated on serine 139 (γ-H2AX) in response to DNA damage and has been considered a specific marker of induction of DSBs. Using flow cytometry, we quantified the γ-H2AX in HKC-8 cell line following exposure to single megavoltage (6 MV) x-ray radiation doses (0 to 10Gy). HKC-8 cells are a human-derived renal proximal tubular cell line and provide a useful model system for the study of human renal cell function. After irradiation, the γ-H2AX formation and loss was analyzed in HKC-8 cells at different times until 24h. Cell cycle phases were also studied by DNA content evaluation. Results and Discussion: The inductivity of γ-H2AX detection by IR, measured in terms of medium intensity of fluorescence (MFI), increased with dose. It presented its maximum value as early as 1hour, (0Gy vs 2Gy: P=0.001; 0Gy vs 4Gy: P<0.001; 0Gy vs 6Gy: P<0.001; 0Gy vs 10Gy: P=0.044) having decreased with time after that, as damage was being repaired. Residual level of γ-H2AX at 24h was lower in 0Gy condition than in irradiated cells, with a significant dose-effect relationship (0Gy vs 2Gy: P=0.515; 0Gy vs 4Gy: P=0.050; 0Gy vs 6Gy: P=0.014; 0Gy vs 10Gy: P<0.01). Additionally, flow cytometry analysis showed that exposure to IR leads to G2 arrest after 24 hours (P<0.001). The preliminary results of this study showed that γ-H2AX expression, as well as its residual level at 24h (which have been suggested as a biomarker of cell sensitivity to killing by IR) are significantly associated with dose. As a consequence of DNA damage, cell cycle is arrested, possibly leading to apoptosis. The sensitivity and reproducibility of γ-H2AX detection by flow cytometry, will enable us to further investigate its relationship with cell cycle events in the study of normal renal response to IR.

No conflict of interest

H3. RADIUM-223: A NEW OPTION FOR METASTATIC CASTRATION-RESISTANT PROSTATE CANCER

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Introduction: Prostate cancer (PCa) mortality are typically the result of metastatic castration-resistant prostate cancer (mCRPC). Historically median survival for men with mCRPC has been less than two years. Recently, the first-in-class alpha-emitting radiopharmaceutical Radium-223 (223Ra) is being considered as the best option treatment for these patients. 223Ra acts as a calcium mimetic by forming complexes with bone mineral hydroxyapatite in areas of high bone turnover, thereby directly targeting areas of bone metastases. The radiopharmaceutical 99mTc- HMDP is also an analog of calcium used in bone scintigraphy. Given the relative lack of information of the molecular pathways responsible for the effects of 223Ra in cells of mCRPC, this project aims to study the effects of 223Ra in PCa cell lines, using as controls one osteosarcoma cell line and the influx and eflux profile of 99mTc-HMDP. MATERIALS AND METHODS: Three tumor cell lines PC3 (metastatic PCa), LNCaP (no metastatic PCa) and MNNG-HOS (osteosarcoma) were incubated with the 223Ra (0.5 µCi/mL) or 99mTc-HMDP (25 µCi/mL). For uptake studies samples of 200 µL were taken at 5, 30, 60, 90, 120, 150 and 180 minutes. Retention studies were performed after cells irradiation for appropriate times. To stop this process, cell suspension was centrifuged and medium changed. After this, samples of 200 µL were taken at 5, 30, 60, 90 and 120 minutes. These were centrifuged to separate supernatant and pellet fractions. Uptake and retention percentage of 223Ra and 99mTc-HMDP was determined after measuring the radioactivity of both fractions in a well counter, in counts per minute. RESULTS AND DISCUSSION: Results show that, at 120 minutes, the percentage of 223Ra uptake by PC3 is twice higher (1,6±0,1%) than by MNNG-HOS (0,8±0,13%). A similar uptake profile was obtained for LNCaP cells (1,26±0,16%). The uptake of 223Ra by MNNG-HOS is five times higher (1,0±0,1%) than the uptake of 99mTc-HMDP (0,2±0,09%). In PC3 cell line the same is verified. By the other side, LNCaP cell line showed an uptake profile similar between two radiopharmaceuticals. Preliminary retention studies show that retention percentage of 223Ra is higher for all cell lines, comparatively to 99mTc-HMDP. These preliminary results suggest that, although both radiopharmaceuticals are analogues of calcium, the uptake and retention mechanisms may be different. This study also enhances the therapeutic potential of 223Ra in the treatment of metastatic PCa. The authors would like to thank Foundation for Science and Technology (FCT) (Strategic Project CNC.IBILI: UID/NEU/04539/2013), COMPETE-FEDER for financial support.

No conflict of interest

H4. The role of APE1 rs1130409 in Breast Cancer susceptibility to acute Radiotherapy side effects

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Introduction: Breast cancer (BC) is the second most common cancer in whole world and appears as the most frequent cancer in women (1.8 million). Breast-conserving surgery followed by Radiotherapy (RT) is the most common treatment for breast cancer. RT is a therapeutic approach that can destroy tumor cells, delivering beams of ionizing radiation and results in the production of a variety of ionizing radiation-induced lesion in DNA. In the post-genomic era, the interest is focused on the phenomenon of inter-individual variation in the response of tumor and normal tissue to RT. The single nucleotide polymorphisms (SNPs) in APE1 may modify individual susceptibility to radiation exposure, which contributes significantly to the increased amount of unrepaired DNA damage, resulting in increased frequency of mutations and genetic instability, as well as change the clinical outcome. The APE1 is essential in the pathway BER, being primarily responsible for the recognition and incision of apurinic/apyrimidinic sites in the DNA fragments damaged by radiation, also presents an important redox modulating function, allowing the maintenance of various cellular transcription factors. In the functional SNP in the fifth exon 2147 T/G (rs 1130409), the G allele may have altered endonuclease and decrease capacity to repair DNA oxidative damage. The aim of this study was to evaluate the influence of APE1 rs1130409 in BC susceptibility to acute RT side effects. Materials and Methods: The group of Caucasian patients (n=94) admitted to the IPO-Porto diagnosed with BC and submitted to RT, have been retrospectively recruited to the study (2009-2011). DNA was extracted from peripheral-blood samples. The APE1 rs1130409 was genotyped by Real-Time PCR. The following clinical skin reactions within the radiation field of the breast were documented during treatment: faint erythema, moderated erythema and moist

desquamation. Acute RT side effects in BC patients were the endpoint of this analysis and were analyzed according to polymorphism genotypes. Results and Discussion: In this study, 16 patients presented no alterations or faint erythema and GG genotype; 1 individual had moderated erythema or moist desquamation and GG genotype. On the other hand, 46 patients had no alterations or faint erythema and GT/TT genotype; 31 individuals had moderated erythema or moist desquamation and GT/TT genotype. According to the results, patients with GG genotype had a statistically significant protection against more aggressive acute RT side effects (moderated erythema or moist desquamation), when compared with GT/TT genotypes patients (P=0.006; OR=0.09; CI95%=0.095-0.73). To properly assess the value of pre-treatment genotyping approaches, prospective collection of genomic DNA from patients with BC should be planned to develop personalized RT protocols for both sensitive and resistant patients.

No conflict of interest

I1. Integrative differential allelic expression analysis efficiently reveals the biology underlying risk to breast cancer

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Introduction Breast cancer is the most common cancer affecting women in the developed world. However, the current knowledge of breast cancer genetic risk cannot explain as much as two-thirds of cases. While successful, GWAS have not: (1) contributed to explain the genetic causality of risk; (2) provided an indication of the biological mechanisms involved. Materials and Methods Based on our previous results demonstrating that cis-regulatory variation is involved in breast cancer risk, we have performed whole-genome differential allelic expression (DAE) analysis of sixty-four normal breast tissue samples. We integrated this genome-wide DAE map with published breast cancer GWAS risk loci according to chromosome location, and linkage disequilibrium between DAE SNPs and GWAS associated SNPs. Results and Discussion We found 38 loci out of 219 (17.35%) displaying GWAS associated SNPs in strong linkage disequilibrium (r2>0.8) with SNPs displaying DAE. Potential regulatory SNPs in top two candidate loci are being currently mapped and functionally studied. We are now integrating the DAE whole-genome map with unpublished GWAS data, to test the efficiency of this approach to help prioritise loci for further risk-association validation. The large overlap between GWAS and DAE data confirms the importance of cis-regulation in the biology of breast cancer risk and provides a new powerful tool to prioritise and functionally analyse risk loci identified through GWAS.

No conflict of interest

J1. Familial Intestinal Gastric Cancer: looking for the causative genes

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Introduction: Ten percent of all gastric cancers (GC) show familial aggregation and may account for at least three syndromes: hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal gastric cancer (FIGC). Whilst germline defects have been found for HDGC and GAPPS families, FIGC remain genetically unexplained. This study aimed at identifying the germline cause in FIGC families and at characterizing the 2nd hits in potentially causative genes occurring in FIGC tumours. Material and Methods: Germline and tumour DNA from 53 FIGC probands were screened using multiplexed targeted sequencing for 55 cancer-associated genes with Illumina's TruSeq Custom Amplicon assay on the MiSeq platform. Selected genes have been implicated in upper gastrointestinal tract cancers or syndromes. Somatic 2nd hits, such as a 2nd mutation, loss of heterozygosity (LOH) and promoter methylation, were searched for in FIGC tumours at potentially causative genes. Results and Discussion: Germline mutations were found in 9/53 (17%) FIGC families fulfilling IGCLC criteria (1- at least 3 relatives with intestinal GC and one should be a 1st-degree relative of the other two; 2- at least 2 successive generations should be affected; 3- GC should be diagnosed before the age of 50 in one relative). These 9 germline mutations were identified in 7 different genes: STK11, SDHB, PRSS1, BRCA2, MAP3K6, MCCC1, MSR1. Four genes were particularly relevant and were further characterized for the presence of 2nd hits. The STK11 (F354L) missense mutation was identified in 2/53 families, and in 1 family, LOH was identified as 2nd hit. The MAP3K6 frameshift (F849*) and PRSS1 nonsense (Q86X) mutations were found in one family each. Germline mutations in these 3 genes were previously reported in HDGC families, highlighting their importance in GC independently of the histological type. One family displayed a novel BRCA2 (K936*) mutation, which is potentially pathogenic due to its gene position. In conclusion, this study identified 4 potentially causative gene defects in FIGC families, highlighting a potential molecular diagnosis for this syndrome. This work is funded by: 1) FEDER/COMPETE, FCT/MEC/FEDER/PT2020 and FCT funds (projects "PEst-C/SAU/LA0003/2013"; project 007274 (UID/BIM/04293); 2) ON.2–O Novo Norte/FEDER/QREN (projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067); 3) No Stomach for Cancer Foundation; 4) FCT Fellowships (SFRH/BPD/89764/2012 to PO; SFRH/BPD/86543/2012 to JC; SFRH/BPD/79499/2011 to HP)

No conflict of interest

J2. Characterization of genetic and clinical aspects of hdgc families from northern portugal

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) is an autosomal dominant syndrome, caused by germline CDH1 mutations that predispose for diffuse type gastric cancer (DGC) and lobular breast cancer (LBC). A third of all described CDH1 mutations are recurrent in HDGC families. However, a founder effect has only been reported for the c.2398delC mutation affecting a large Newfoundland family. We identified 5 seemingly unrelated families, originating from the North of Portugal, carrying the c.1901C>T CDH1 mutation, which are followed at the CHSJ-Porto. We aimed at characterizing these families both genetically and clinically, and determining whether this recurring mutation presents a common ancestry. Material and Methods: We collected family history and clinical data, performed c.1901C>T carrier tests, and carried out haplotype-based analysis through genotyping of flanking microsatellite markers and 2 single nucleotide polymorphism (SNPs) within the CDH1 gene. Results and Discussion: We studied 73 individuals from 5 families and verified that 39/73 (53%) carried the c.1901C>T mutation (age range: 14-82 years). Seventeen of the 39 (43.6%) carriers were either affected by/died of DGC or LBC cancer (10/39 - 25.6%; age range: 18-62 years), or revealed cancer foci in prophylactic gastrectomy specimens (7/39 - 17.9%; age range: 14-66 years). From the 10 patients affected by cancer, 3 (30.0%) had LBC at the age of 52, 61 and 62, while 7 (70.0%) had DGC (age range: 18 to 35 years). Importantly, DGC occurred very early in the life of mutation carriers, with the youngest dying of cancer at 18, and cancer being found in a prophylactic gastrectomy from the 14-year old carrier sib, while LBC occurred always after 50. These results suggest that genetic testing should not be limited to at risk relatives of 18 plus years, and should be made available sooner, after proper genetic counseling. Haplotype analysis performed in mutation carriers from at least 2 generations in each family revealed a unique haplotype, indicating a common ancestor, rather than independent mutational events. Two new families carrying this same mutation and from the same geographical region, have been identified at IPO-Porto, and will be included in the study. Our findings highlight the need for DGC surveillance in young carriers of the CDH1 c.1901C>T mutation, and provide the rationale for prioritizing the screening of this mutation in families from this geographical area. Support: 1) FEDER/COMPETE, FCT/MEC/FEDER/PT2020 and FCT funds (projects "PEst-C/SAU/LA0003/2013"; project 007274 (UID/BIM/04293); 2) ON.2–O Novo Norte/FEDER/QREN (projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067); 3) No Stomach for Cancer Foundation; 4) FCT Fellowship SFRH/BPD/79499/2011 to HP.

No conflict of interest

K1. Novel cell models to study tumour-stroma interactions and disease progression mechanisms in vitro

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Introduction: Tumour microenvironment is composed of a network of fibroblasts, endothelial and immune cells embedded in the extracellular matrix under specific physicochemical conditions (e.g., hypoxia and acidic pH). Together, these components influence tumour progression and drug resistance through tumour-stroma crosstalk. Numerous 3D tumour cell models have been established in recent years aiming to reflect the high complexity of the tumour microenvironment and to mimic tumour progression. However, most of these models which are generated in culture systems that do not allow long-term culture, continuous monitoring and often use bioactive scaffolds. Materials and Methods: Our strategy is based on stirred-tank culture systems and alginate microencapsulation. Several components of tumour microenvironment were incorporated, such as stromal cells (fibroblasts and monocytes) and specific physico-chemical parameters. Breast and lung tumour cell aggregates were microencapsulated in alginate alone or in combination with stromal cells and cultured for up to 20 days. Results and Discussion: Microencapsulation of tumor spheroids with stromal cells allowed the establishment of distinct epithelial and stromal compartments. MCF7, an oestrogen Receptor (ER) + breast tumour cell line, established cell-cell contacts and polarised around small lumina in the interior of the aggregates, recapitulating the in vivo tissue organization. Over the culture period fibroblasts secreted collagen into the stromal compartment and the presence of both fibroblasts and monocytes resulted in a pro-inflammatory environment. This was accompanied by a reduction of ER and membranous E-cadherin alongside loss of cell polarity, increased collective cell migration and enhanced angiogenic potential only in co-cultures. These phenotypic alterations are typical of advanced stages of cancer. In contrast, the effects of fibroblasts were not as significant in NSCLC using H1650, H1437 and H157 suggesting that the effect of tumor-stroma cross-talk is cell line dependent. Moreover, treatment with fulvestrant, an ER antagonist, reduced cell concentration in mono-cultures but not in co-cultures although the number of proliferating cells was reduced in both. In summary, we developed a robust model system for long-term in vitro recapitulation of tumour-stroma crosstalk and monitoring of disease progression, applicable to several pathologies. This constitutes a new tool for characterization of disease progression and drug resistance mechanisms in vitro. Acknowledgements: We acknowledge Dr. Cathrin Brisken, Dr. Heiko van der Kuip and Dr. Moshe Oren for the supply of the cell lines. This research received support from the Innovative Medicines Initiative Joint Undertaking (grant agreement n° 115188), FCT (iNOVA4Health—UID/Multi/04462/2013). MFE is recipient of a PhD fellowship from FCT SFRH/BD/52208/2013.

No conflict of interes

K2. Effect of surface functionalization of zeolite L on cellular uptake in breast cancer and epithelial mammary cells

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Introduction: During the last decade, nanomaterials have been extensively used for drug delivery[1], because of that the understanding of the interactions of nanomaterials with cells has been a focus of interest.[2] Zeolites are solid inorganic crystalline nanomaterials, which have biomedical applications such as drug delivery systems (DDS).[3] The functionalization of the zeolite surface can enhance the intracellular delivery of drugs.[4] In this work, we investigated the cytotoxicity and uptake of zeolite L unmodified and modified with NH2, COOH groups and PLL in order to evaluate if the surface functionalization improves cellular uptake. Materials and Methods Zeolite L was functionalized with amino groups (L-NH2), carboxylic groups (L-COOH) and coated with poly-L-lysine (L-PLL). The toxicity of modified zeolites was then evaluated on breast cancer cells (Hs578T) and epithelial mammary cells (MCF-10) by preparing six zeolite concentrations from 10 µg/mL to 125 µg/mL. To investigate the cellular uptake of unmodified and modified zeolite, we used a variety of techniques such as confocal microscopy (CLSM) and scanning electron microscopy (SEM). Results and discussion Toxicity studies show that the viability of Hs578T cell line decreased with increasing material concentration. Instead MCF-10 cells growth is not affected even at 125 µg/mL. In the cellular uptake experiments, cells internalized some particles 5 min after starting the incubation and the amount of internalized particles increased over time. Importantly, L-NH2- and L-PLL zeolites presented faster internalization over the studied period time, compare to zeolite L or L-COOH zeolite. These results can be explained by the fact that the positively-charged surface allows electrostatic interactions with the negatively-charged cellular membranes, favoring cellular uptake. Based on these results we can attest the potential of modified zeolites as nanocarriers for drug delivery. Acknowledgements: N.V., F.S. and R.A. are recipients of PhD fellowships (SFRH/BD/97797/2013, SFRH/BD/87139/2012, SFRH/BD/98002/2013, respectively) from Fundação para a Ciência e a Tecnologia (FCT, Portugal). The research centers CQ/UM and ICVS/3B's are financial supported by FCT, FEDER-COMPETE-QREN-EU. Reference [1] K. Park. J Control Release. 206 (2015) 243. [2] D.F. Moyano, et al. Langmuir 27 (2011) 10376-10385. [3] N. Vilaça, et al. Colloids and Surf B 112 (2013) 237-244. [4] J.M. Steinbach, et al. Acta Biomaterialia 30 (2016) 49-61.

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K3. Fab-conjugated PLA nanoparticles for dendritic cell targeting

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Introduction: Dendritic cell (DC)-based cancer vaccines are expected to elicit a specific immune response against tumor cells. It is thus important to design an efficient platform able to target DCs and modulate the intracellular trafficking of antigens [1]. Based on previous work [2], PLA-PEG-bis-sulfone polymeric nanoparticles (NPs) that can be site-specifically functionalized with antigen-binding fragments (Fabs) were developed to accomplish specific targeting of DCs. Materials and Methods: PLA-PEG-bis-sulfone polymer was synthesized by covalent coupling of bis-sulfone-PEG-amine with N-hydroxysuccinimide PLA ester. Preliminary characterization of PLA-PEG-bis-sulfone was performed through proton NMR and FT-IR spectroscopy. To assess the ability of PLA-PEG-bis-sulfone to conjugate with Fabs, reduced Fabs were added to a solution of PLA-PEG-bis-sulfone and incubated at room temperature overnight. SDS-PAGE electrophoresis was performed to evaluate the conjugation. PLA-PEG-bis-sulfone was then used to formulate NPs by state of the art double emulsion solvent evaporation method, using PVA as surfactant and a mixture of PLA-PEG-bis-sulfone and PLA (1:9). NPs were recovered by centrifugation and washed to remove excess of surfactant. mPEGPLA/PLA NPs (1:9) were used as control. The mean diameter and polydispersity index (PdI), surface charge and morphology of NPs were analyzed through DLS, zeta potential and TEM, respectively. Finally, reduced Fabs were allowed to react with bis-sulfone moieties of PLA-PEG-bis-sulfone NPs, under gentle stirring overnight. The association of Fabs to PLA-PEG-bis-sulfone/PLA NPs was assessed by Ellman's reagent assay. Results and Discussion: Proton NMR and FT-IR analysis proved that PLA-PEG-bis-sulfone was synthesized. Characteristic NMR shifts were observed at 1.58 – 1.64 ppm, corresponding to PLA, 3.48-3.7 ppm of PEG moiety and the distinctive signal of the bis-sulfone group between 7 and 8 ppm. PLA-PEG-bis-sulfone was found able to conjugate with reduced Fabs, which was concluded from SDS-PAGE gel analysis by the presence of a band between 65 and 70 kDa, corresponding to PLA-PEG-bis-sulfone (12kDa) conjugated with Fab (50 kDa). mPEG-PLA/PLA (1:9) and PLA-

PEG-bis-sulfone (1:9) formulations both have led to spherical NPs with approximately 250 nm in diameter (PdI<0.2). Surface charge of NPs was neutral, which is important to decrease electrostatic interactions of Fabs and NP surface. The association of Fabs to PLA-PEG-bis-sulfone NPs was assessed using Ellman's reagent, in a preliminary assay. The association of Fabs to PLA-PEG-bis-sulfone NPs was approximately 80% and superior to the determined for mPEG-PLA/PLA NPs, used as negative control. This work showed that PLA-PEG-bis-sulfone NPs are a suitable strategy for conjugation of Fabs, presenting promising results. The method will be further characterized and optimized. At the end, NPs will be used for DC targeting in order to potentiate antigen delivery to these phagocytic cells, which will present them to T cells, promoting tumor cell eradication.

No conflict of interest

K4. Nanoparticle-based cancer vaccine to deliver tumor associated antigens and for immunomodulation

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Cancer vaccines have been used as an alternative therapeutic strategy and have already shown promising results. However, only a small number was able to lead to an effective tumor regression, which can be explained by the immunosuppressive properties of tumor microenvironment induced namely by the release of potent immunosuppressor molecules. Therefore, the elimination of both the tumor itself and the tumor microenvironment, without adversely affecting the desired antitumor effector cells, seems to be an ideal therapeutic strategy to eradicate this disease. Thus, the aim of the present study is to develop a polymeric nanoparticle (NP)-based cancer vaccine to deliver incorporated tumor-associated antigens and/or small interfering RNA (siRNA) to target dendritic cells (DCs) and for immunomodulation by silencing immune-suppressive cytokines within breast tumor site. Antigen or siRNA-chitosan complexes encapsulated in poly(lactide acid) (PLA) NPs have been formulated by a double emulsion solvent evaporation method. These NPs were coated with polyvinyl alcohol (PVA) or with block co-polymer Pluronic to improve stability under physiological conditions. In order to potentiate tumor targeting, NP surface was modified by hyaluronic acid (HA), a targeting moiety that specifically recognizes CD44 receptor, overexpressed on several tumor cells. NP size, surface charge (ZP) and morphology were analyzed by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy (AFM), respectively. Antigen entrapment efficiency (EE) and loading capacity (LC) were quantified by HPLC, while siRNA EE and LC were determined by PicoGreen® reagent. Finally, cell viability was determined by Alamar Blue® assay. siRNA-NP knockdown capacity is currently under evaluation by Western blotting and flow cytometry. Overall, NPs presented a mean diameter close to 200 nm with low polydispersity index (PdI) values (≤0.200), ZP close to neutrality, and high EE (>85%) values for both antigen and siRNA. PLA NPs showed no cytotoxicity on targeted cells and DCs after 72h of incubation, even at high NP concentration 0.5 mg/mL). Three different chitosan (Cs) derivatives were used for antigen or siRNA complexation. However, no significant differences were observed between the physicochemical properties of those three different nanoparticulate systems. Similarly, no significant differences were detected in NP's size, surface charge and cytotoxicity when formulated with PVA and Pluronic as external phase surfactant. Moreover, non-targeted and targeted NPs also presented similar properties. Therefore, it is possible to state that the formulation method followed for PLA-based NP preparation is highly reproducible and this nanoparticulate system constitutes a promising platform for the delivery of TAA and immunomodulators to different cells within tumor microenvironment.

No conflict of interest

K5. LDL inhibits the anti-tumor functions and breast cancer targeting by γδ T cells

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Introduction: γδ T cells are known to efficiently recognize and kill a variety of tumors in vitro, including breast cancer cell lines. However, the systemic or local factors that may regulate anti-tumor γδ T cells remain poorly understood. Recently, high LDL-cholesterol was shown to perturb the bone marrow microenvironment and promote bone marrow cell mobilization. Aims: This study aims to understand if and how systemic LDL-cholesterol impacts the capacity of γδ T cells to recognize and eliminate tumors, namely breast cancer. Methods: Freshly isolated γδ T cells were enriched with interleukin-2 (IL-2) and (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) during 12 days. These cells were tested for activation (CD69 expression), cytotoxicity receptors (NKG2D, DNAM and CD56) levels, intracellular cytokine production (IFN-gamma, TNF-alpha and IL-17), and killing assays against the breast cancer cell line, MDA231. Results: In presence of LDL-cholesterol, the activation and anti-tumor functions of γδ T cells were significantly inhibited, as illustrated by decreases in the following parameters: CD69 expression, intracellular IFN-γ production, NKG2D, DNAM-1 and CD56 (identifies highly cytotoxic cells) levels; and in vitro killing of MDA231. Our study describes for the first time, to our knowledge, the role of LDL-cholesterol as an important inhibitor of antitumor γδ T cell functions.

No conflict of interest

K6. Major quality parameters of long-term cryopreserved cord blood units - a single center experience

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Introduction: Cord Blood (CB) is a well-recognized source of stem cells for hematopoietic transplant. Its main advantage is to be stored for long-term with a ready availability for the intended recipient and no risk for the donor. The aim of this study was to evaluate the quality of 15 cord blood units cryopreserved in our direct donation bank. Materials and Methods All these units were processed without red cells reduction, in a 24 hours period after collection, then were cryopreserved with a controlled-rate freezer and transferred to a liquid nitrogen container. We used cryogenic bags (Kapton/Teflon), highly resistant to very low temperatures. However, due to missing data we do not considered these CB units suitable for transplant. In this study, the CB were thawed and washed based on the New York Blood Center method in our ISO 7 cleanroom, inside of an air flow cabinet. We performed cellular counts, viability assays (by flow cytometry using 7-aminoactinomycin), sterility testing (bacterial and fungal cultures) and clonogenicity (colony-forming units-granulocyte/macrophage - CFU-GM - growth). The correlation between CD34+ cell and CFU-GM counts was evaluated by linear regression analysis. Results and Discussion The CB units were stored during 11-20 years (mean 16±3). We analyzed 12 CB because 3 bags were broken during storage (20%); this percentage is much higher than that found in our daily practice (4%), which may be caused by the long cryopreservation period. Visual examination of the product showed evidence of hemolysis in 10 CB and fibrin clots in 1. The recovery of total nucleated cells (TNC) was 73±14%; CD34+ cells enumeration 48.3±29.3x10⁵ and CFU-GM quantification 28.5±19.3x10⁴. The TNC and CD34+ cells viability was 70±8% and 91±5%, respectively. We obtained a strong positive relationship between CD34+ cell and CFU-GM (R2=0.758). We found 4 contaminated CB, one of them was already positive after processing (Enterococcus faecalis, Escherichia coli and Streptococcus mitis). We hypothesize that the pre-freezing hemocultures were not representative of the product; however, we cannot exclude a cross-contamination during the storage. Despite our small serie, the results of cellular viability, purity and potency indicate that long-term cryopreservation does not negatively affect the quality of CB units for further use, even in the presence of contamination, hemolysis signs and aggregates. We think that every cord blood bank should have an expert to help transplant physicians select the best cord blood for his patient based on the control quality results performed before final release.

No conflict of interest

K7. Monitoring minimal residual disease in autologous cellular therapy products collected by apheresis – experience of a center

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Introduction: The autologous transplant of peripheral blood stem cells (PBSC), harvested by apheresis on oncologic patients after high-dose chemotherapy and/or radiotherapy, is a therapeutic strategy used in hematologic diseases and solid tumors. Some studies suggest that incomplete disease eradication before collection could lead to the reinfusion of tumor cells and be associated with patient relapse and a lower survival. The search for minimal residual disease (MRD) has been performed in some centers through highly sensitive genetic techniques, contributing to improve the clinical decision and the patient outcome. This study aims to evaluate the percentage of MRD-contaminated grafts in patients proposed to hematopoietic transplant with initial genetic alterations in our hospital. Material and Methods A retrospective study was performed from May 2006 to February 2016. The inclusion criteria was patients with genetic alterations at diagnosis or relapse. The research was performed using fluorescent in situ hybridization (FISH) or/and real time polymerase chain reaction (RT-PCR) techniques. The data was analysed using an excel program. Results and Discussion MRD was researched in samples of PBSC collected from 39 male and 28 female patients (n=67), with a median age of 51 years old (7 months – 70 years). Their diagnosis were: non Hodgkin's lymphoma (NHL) n=24, multiple myeloma (MM) n=16, neuroblastoma n=17, acute myeloid leukemia (AML) n=5, acute lymphoblastic leukemia (ALL) n=2, Hodgkin's disease (HD) n=2 and Ewing's sarcoma (Ewing S.) n=1. Only 6 grafts were MRD positive, but with a very low tumour cell contamination (1-2%): 1 AML with PML-RARA fusion RNA; 1 ALL with BCR-ABL mRNA; 2 MM with deletion of 13q14; 1 Ewing S. with translocation of 22q12; 1 neuroblastoma with deletion of 11q23. Three contaminated grafts were eliminated: 2 patients (1 AML and 1 neuroblastoma) were proposed to different chemotherapy lines, undergone new mobilization and collection program and received a negative cellular therapy product; the ALL patient died before the second mobilization. Two MM patients were infused with the autologous grafts. In the remaining patient (Ewing S.), we performed a positive selection of CD34+ cells after thawing and before graft infusion. At present, 2 patients are alive and in complete remission, 9 and 10 years after treatment (Ewing S. and AML); 3 patients deceased 1, 7 and 4 years after transplant (2 MM and 1 neuroblastoma, respectively). As it was a very small and heterogeneous sample, conclusive remarks of the role of MRD significance in stem cells grafts are not possible. In the same way, we only performed one ex vivo tumoral purging with identical results to those found on literature. Future prospective trials should address physicians to choose which is the best option to eradicate tumor cells (chemotherapy pretransplantation or immunotherapy postransplantation).

No conflict of interest

K8. Breast cancer biomarker analysis using a molecularly imprinted polymer sensor

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Introduction: Cancer antigen 15-3 (CA 15-3) is one of the circulating biomarkers of breast cancer. The development of a point-of-care system for the analysis of this biomarker could be very important to evaluate the patient's response to treatment and/or to evaluate the recurrence/progression of the cancer. For this purpose molecularly imprinted polymer (MIPs) sensors are excellent candidates because of their high sensitivity, selectivity, fast response, simplicity, low cost and easy miniaturization. These sensors can be prepared though surface imprinting of the target analyte directly on the sensor. In this work an electrochemical MIP sensors was prepared for the analysis of CA 15-3. Materials and Methods For the development of the MIP sensor a screen-printed gold electrode was used. Initially CA 15-3 was adsorbed onto the electrode's surface and a polymer was formed around the protein by electropolymerization. After these steps the protein was extracted from the polymer, forming cavities for selective binding of CA 15-3. The sensor could then be applied to the analysis of CA 15-3 through (i) incubation with a CA 15-3 containing sample and (ii) subsequent voltammetric measurements. This analysis was performed using differential pulse voltammetry (DPV), using

[Fe(CN)6)]3-/4- as redox probe, measuring the differences between the signals before and after protein binding. Electrochemical impedance spectroscopy and cyclic voltammetry were used for the characterization of the developed sensor. During the development of the MIP sensor the following conditions were optimized: the used monomer, the electropolymerization process, the extraction solvent and time, and the DPV parameters. Results and Discussion A linear relationship between the logarithm of the CA 15-3 concentration and the analytical signal was established between 10 and 60 U/mL. A limit of detection of 1.5 U/mL was achieved. The obtained results indicate that the developed MIP sensor could be a promising tool in breast cancer management. Nevertheless, further studies are being conducted using spiked serum samples and in a later stage patients' serum samples will be analyzed. Acknowledgments This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e a Tecnologia) through project UID/QUI/50006/2013. João.G. Pacheco is grateful to FCT for his Pos-Doc grant (SFRH/BPD/101419/2014).

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