One Day Symposium sponsored by EACR

Darwinian evolution and clonal heterogeneity in human cancer: biological and clinical implications

Keynote Speaker Carlos Caldas

Monday 29th October, 2012

Fundação Eng. António de Almeida

Porto, Portugal

Scientific Organising Committee

Leonor David • Carmen Jerónimo • Fátima Baltazar

Fátima Cardoso • Raquel Almeida • Luis Costa

Proceedings

Programme

Monday 29th October 2012

8.30 – 9.00

Registration at Fundação Eng. António de Almeida

9.00 – 10.00

Plenary Session 1

Chairs: Manuel Teixeira (IPO-Porto/ICBAS and Alexandre Quintanilha IBMC/ ICBAS, Porto)

9.00 - 9.15

Tumour banks in Portugal

Fátima Carneiro (FMUP/Hospital S.João/IPATIMUP, Porto)

9.15 - 9.30

The ROR-Sul new platform, an innovative tool for cancer research and practice development

Ana Miranda (IPO-Lisbon)

9.30 - 9.45

PEM in breast cancer

João Varela (IST/LIP, Lisbon)

9.45 – 10.00

From preclinical and clinical cancer diagnostic and screening to real-time in vivo dose monitoring for assisting external beam radiotherapy: an overview of the medical imaging activities and radiobiological plans at LIP Coimbra and collaborations

Paulo Crespo (Universidade Coimbra/ LIP, Coimbra)

10.00 - 10.30

Coffee break

10.30 – 11.30

Plenary Discussion on platforms, facilities and technologies

Chairs: José Mariano Gago (IST/LIP, Lisbon) and Manuel Sobrinho Simões (IPATIMUP/FMUP/Hospital S.João, Porto)

11.30 – 13.00

Plenary Session 2: Keynote Lecture

Chair: Julio Celis (EACR)

Darwinian evolution and clonal heterogeneity in human cancer – biological and clinical implications

Carlos Caldas (Cambridge Research Institute, UK)

13.00 - 15.00

Lunch and Poster Viewing

Julio Celis (EACR), Carlos Caldas (CRI), Sergio Dias (IMM/FMUL/IGC, Lisbon), Carla Oliveira (IPATIMUP/FMUP, Porto), Ana Teresa Maia (UA, Algarve), Luis Costa (FMUL/IMM, Lisbon), Carmen Jerónimo (IPO-Porto/ICBAS, Porto), Fátima Baltazar (ICVS, Braga)

15.00 – 16.20

Plenary Session 3

Chairs: Fátima Carneiro (FMUP/Hospital S.João/IPATIMUP, Porto) and Sergio Dias (IMM/FMUL/IGC, Lisbon)

15.00 – 15.20

Male Germ Cell Tumours diagnosed in 1999 and 2000 - a population-based retrospective study in Southern Portugal

José Luis Passos Coelho (Hospital da Luz/Hospital Beatriz Angelo/FCM-UNL, Lisbon)

15.20 – 15.40

E-cadherin disfunction in gastric cancer. Celullar consequences and clinical applications

Raquel Seruca (IPATIMUP/FMUP, Porto)

15.40 – 16.00

From the environment, from within: IL-7R-mediated signaling in T-cell leukemia

João Barata (IMM, Lisbon)

16.00 – 16.20

Telomerase is required for melanoma progression

Miguel Godinho (IGC, Oeiras)

16.20 - 17.00

Coffee break

17.00 – 17.20

Young Investigators Awards Presentations

17.20 – 18.20

Address by Julio Celis, EACR Past President, and Meeting of the Portuguese Division of EACR

Julio Celis, Manuel Sobrinho Simões and the Steering Committee of the Portuguese Association for Cancer Research (Leonor David, Carmen Jerónimo, Fátima Baltazar, Fátima Cardoso, Raquel Almeida, Luis Costa and Rita Barros)

About the Keynote Lecturer: Carlos Caldas

Carlos Caldas is the leader of the Breast Cancer Functional Genomics Laboratory at the Cancer Research UK Cambridge Research Institute, which he joined in 2006. The main research interest of Carlos is breast cancer and more specifically to understand how genetic alterations accumulate, how they determine the biology of cancers, and which cell population within the breast epithelium is targeted by these alterations.

His group has identified novel cancer genes, has functionally characterized cancer associated genes and validated human breast carcinoma prognostic/predictive/therapeutic targets. The molecular profiling and description of the complex molecular taxonomy of breast cancer is the first step towards robustly identifying markers that have true clinical utility. Four Nature papers among other top journal publications on this subject were published in 2012.

EACR

Summer Conference 2013

25 - 28 June 2013
Cambridge, U.K.

First Announcement

EACR Summer Conference: Cancer Genomics

25 – 28 June 2013

Churchill College, Cambridge, U.K.

Organisers

James Brenton, UK • Carlos Caldas, UK

Jessica Downs, UK • George Vassiliou, UK

Keynote Speakers

Sam Aparicio, Canada • Shankar Balasubramanian, UK • Anne-Lise Borresen-Dale, Norway

Invited Speakers

Rene Bernards, the Netherlands • James Brenton, UK • Carlos Caldas, UK

Luis Alberto Diaz, USA • Manel Esteller, Spain • Gad Getz, USA

Mel Greaves, UK • Sean M. Grimmond, Australia • Jos Jonkers, the Netherlands

Jan Korbel, Germany • Peter Lichter, Germany • Elaine Mardis, USA • Charles Perou, USA

Martin Peifer, Germany • Nitzan Rosenfeld, UK • George Vassiliou, UK

Important Deadlines

Abstract Submission: 30th April 2013

Meeting Bursary Application: 30th April 2013

Registration: 31st May 2013

EACR Meeting Bursaries

Five bursaries, each of 500 Euros, will be awarded to assist members of the EACR to attend the meeting

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Building a network of tumour banks in Portugal

Fátima Carneiro[1,3]
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Among biobanking initiatives, Tumour Banks play a pivotal role in biomedical research. The general aim of a Tumour Bank is to acquire neoplastic and control non-neoplastic samples, in standardized conditions for research (basic, clinical or translational). A Tumour Bank is a vital new resource for cancer research, providing high quality, well-characterized tissue.

It is possible for pathologists to collect fresh tissue prospectively during their routine dissection procedures. In this way, the specimens can be optimally sampled and stored for both diagnosis and research purposes. Ideally, specimens are sampled immediately after surgery, prior to fixation, to ensure optimal preservation of proteins and nucleic acids. Retrospective collection of tumour tissue for study and banking purposes is feasible also because, in most countries, pathology laboratories have been legally obliged to file, for at least some years, the formalin- fixed and paraffin-embedded samples that were analyzed.

Over the last decade, Tumour banks acquired a pivotal role in translational research in the field of oncology, providing tools for: evaluation of new predictive factors; evaluation of the value of a known target in a new entity; search for new therapeutic targets; validation of new diagnostic markers; implementation of new diagnostic procedures, namely development of tissue-based diagnostic tests for guidance of therapy with new drugs introduced in clinical practice.

In this scenario, it is a priority to emphasize the central role that pathologists play in translational research, specifically in tumor banking, by the establishment of a bridge between clinicians and basic researchers.

In this presentation it will be presented the steps to establish the Tumour Bank of Hospital S.João, as well as the initiatives to build a National Network of Tumour Banks in Portugal [1].

[1] http://www.acs.min-saude.pt/2009/12/18/projectornbt/

The ROR-Sul new platform, an innovative tool for cancer research and practice development

Ana Miranda, *IPO-Lisbon*

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In an effort to fulfil a need on cancer-related information, the South regional Cancer Registry (ROR-Sul) was created in 1988 and regulated by Law.

This is a population-based cancer registry that ensures the active surveillance of all residents from the continental South region and Madeira Island, around 4.8 million inhabitants (4 regions that account for nearly half the country).

The ROR-Sul was the first European registry developed as a network, but currently the new platform is a step ahead, since it is based on a record-linkage system integrating information from various independent data sources. There are essentially three types of information being linked, allowing for an overall picture of the case: patient identification (integrating information from the citizens' card updated every fortnight, e.g. name, age, date of death), diagnostic data (e.g. case definition, pathology results; where all classifications used follow the ENCR recommendations), and treatment data (including surgery, radiotherapy, and chemotherapy; originating from 3 independent databases). This system now allows for a case to be monitored longitudinally from the moment of presentation of first symptom until his death, which has enormous applications.

Confidentiality is not compromised, since there are levels of access defined according to the user profile and information circulates in a private network. This allows clinicians to see the case as a whole with the most up-to-date information, while allowing him to register his own information. The central processing information ensures it is permanently available for research purposes.

In summary this platform may be seen as:

1) An information system, including the management of its quality, a prerequisite to deal with data from various sources.

2) A working tool that allows case registry; online monitoring of case in clinical practice; research on patterns of cancer care, cancer epidemiology and pharmacoepidemiology.

PEM in breast cancer study and diagnosis

Joao Varela[1,2]
[1]*LIP, Lisbon, Portugal,* [2]*IST, Lisbon, Portugal*

Two prototypes of a new Positron Emission Mammography (PEM) scanner developed by a Portuguese consortium, are currently operating at the Institute of Nuclear Sciences Applied to Health (ICNAS), in Coimbra, and at the University Hospital in Marseille. The scanner has millimetric image resolution and high sensitivity allowing precise PET characterization of cancer tumors. Clinical tests with patients affected by breast cancer and patients with other cancer types (control sample) were performed parasitically in cases where a PET exam was prescribed. A summary of the results will be discussed. Results obtained with gelatin phantoms emulating the breast tissue and lesions of different sizes will also be presented. Examples of tests performed with mice will be presented illustrating the potential of this tool in biomedical research.

From preclinical and clinical cancer diagnostic and screening to real-time in vivo dose monitoring for assisting external beam radiotherapy: an overview of the medical imaging activities and radiobiological plans at LIP Coimbra and collaborations

P. Crespo[1,2], F. Alves[3,2], M.C. Battaglia[2,4], V. Bellini[4,5], A. Blanco[1], P. Cambraia Lopes[6,1], M. Capela[7], A. Cavaco[8], S. Carmo[2], M. Couceiro[1,3], N.C. Ferreira[2], R. Ferreira Marques[1,2], P. Fonte[1,3], F. Fraga[1,2], S. Ghithan[1,2], L. Lopes[1], M.C. Lopes[7], P. Martins[1,2], K. Parodi[9,10], P.J.B.M. Rachinhas[8], D.R. Schaart[6], H. Simões[1], P.C.P.S. Simões[8], P. Soares[8]
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The theoretical and technological skills of the high energy physics community often serve those of medical physics. For instance, Monte Carlo simulations for describing particle interactions in physics experiments are now commonly used to characterize and improve modern radiotherapy treatments both with X-rays and with particles such as protons and carbon ions. On the technological side, the demands for developments and improvements of performance of detectors and associated technology also embrace the two aforementioned fields of physics. For these reasons LIP Coimbra is strongly engaged in several projects focusing mainly on medical imaging, but not only. This communication will summarize these projects, namely (1) preclinical and clinical high-sensitivity positron emission tomography (PET) with new, very-high-resolution detectors; (2) developments aiming at real-time, in vivo dose monitoring for assisting (and improving) particle and photon radiotherapy; and (3) towards radiophysiology and radiobiology studies with proton beams at the PET proton cyclotron of the University of Coimbra. A strong emphasis will be put on explaining the basic clinical motivation and physical concepts of these projects.

Male Germ Cell Tumours diagnosed in 1999 and 2000 – a population-based retrospective study in southern Portugal

José Luis Passos Coelho
Hospital da Luz, Lisboa e Hospital Beatriz Angelo, Loures
Faculdade de Ciências Médicas, Lisboa

Results obtained in clinical trials for treatment of oncologic diseases may not be reproduced when the same approach is applied to patients with the same disease in the community. Furthermore, the reality of one country may not apply to a different country despite similar overall population characteristics. This study demonstrates the relevance of characterizing the national reality regarding the clinical presentation and therapeutic outcomes for oncologic diseases. In close collaboration with the regional cancer registry of southern Portugal (ROR-Sul) and of institutions and professionals involved in diagnosis and treatment of germ cell tumours, a retrospective population-based study was undertaken on southern Portugal and Madeira island, including males diagnosed with germ cell tumours in 1999 and 2000; this allowed a minimum follow-up time of 5 years. Eighty seven patients were identified (incidence of 1.85/100.000 males), 79 with primary testicular tumours. From 81 patients with testicular or retroperitoneal tumours, 35 were diagnosed with stage I, 13 with stage II and 30 with stage III (3, stage unknown). With a median follow-up of 89 months, global 5-year overall survival was 80% (100% for stage I, 13 for stage II and 53% for stage III). The results obtained show a similar incidence to other countries of southern Europe but a lower survival than reported in Eurocare4 study (performed in patients diagnosed between 1995 and 1999), with 5 year-overall survival ranging between countries from 92% to 98%. The data suggest a possible delay in diagnosis, with a high proportion of patients diagnosed with advanced stage high-tumor bulk disease, as well as sub-optimal adherence to recommended treatment algorithms. Data like these may be useful to identify limitations in the results of treatment of cancer and should lead to enhanced interest from health care professionals and authorities.

E-cadherin disfunction in gastric cancer. Celullar consequences and clinical applications

Carla Oliveira[1,2], Joana Figueiredo[1], Patrícia Carneiro[1], Joana Carvalho[1], Joana Caldeira[1], Patrícia Oliveira[1], Joana Paredes[1,2], Joé Carlos Machado[1,2], Fátima Carneiro[1,2], Raquel Seruca[1]
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Tissues in multicellular organisms consist of a variety of cells in which cell-cell and cell-matrix adhesions are key events to allow correct tissue architecture and tension. Cell-cell adhesion is mediated by a variety of membrane proteins such as E-cadherin which is the major component of the Adherens Junctions (AJs) and the major contributor to the maintenance of adult tissues integrity and homeostasis. The critical importance of E-cadherin to normal development is demonstrated by the lethality in the very early stage of embryogenesis.

One of the most basic characteristics of cancer cells is that they adhere poorly to each other, being this fact usually associated with their ability to invade the surrounding tissues. In cancer, the study of sporadic tumours and early hereditary diffuse gastric cancer (HDGC) lesions in germline CDH1 mutation carriers suggests that E-cadherin loss can be an early or initiating event in tumorigenesis but also an important marker for therapeutical selection of the patients. To unravel the molecular mechanism underlying the role of E-cadherin in cancer, we have performed several in vitro and in vivo studies (animal models and primary gastric carcinomas). As example, using a set of 42 stable cell lines, harboring HDGC associated E-cadherin germline mutations distributed along the gene, we clarified E-cadherin mediated signaling pathways and associated cellular effects. We demonstrated that E-cadherin in tumor progression depends on the activation of signaling pathways related to migration and cell survival. Further, we identified EGFR and Notch as interesting therapeutic targets in E-cadherin mediated cancer.

From the environment, from within: IL-7R-mediated signaling in T-cell leukemia

João Barata
Instituto de Medicina Molecular, Lisboa, Portugal

Although cancer is a genetic disease, it is currently evident that microenvironmental cues are essential for tumor progression. T-cell acute lymphoblastic leukemia (T-ALL), an aggressive subtype of the most frequent childhood cancer, is no exception. Interleukin 7 (IL-7), a cytokine produced in the bone marrow, thymus and other organs, is mandatory for normal human T-cell development. However, there is also considerable evidence that IL-7 may partake in leukemia development. We showed that IL-7 leads to the activation of PI3K/Akt/mTOR pathway, thereby mediating viability, cell cycle progression and growth of human T-ALL cells in more than 70% of patient samples. Remarkably, the involvement of PI3K/Akt/mTOR pathway in these processes differs subtly between normal and malignant T-cells, in a way that may have important therapeutic implications. We further showed that microenvironmental IL-7 can have a role in accelerating human T-ALL progression in vivo. The evidence that IL-7 produced by the stroma has considerable impact on leukemia maintenance led us to go "back to the basics" and evaluate whether cell-autonomous lesions could affect directly IL-7-mediated signaling in malignant T-cells. We showed that around 9% of T-ALL patients display gain-of-function mutations in the gene encoding the IL-7 receptor (*IL7R*). The mutations lead, in most cases, to disulfide bond-dependent homodimerization of two mutant receptors and consequent constitutive activation of downstream signaling, with ensuing cell transformation *in vitro* and tumorigenic ability *in vivo*. Is the oncogenic potential of deregulated IL7R-mediated signaling restricted to mutated receptor? Our studies showing that conditional tetracycline-inducible IL7R transgenic mice eventually develop leukemia upon treatment with doxycline, suggest otherwise. Overall, our results revealed IL-7/IL-7R-mediated signaling as an important oncogenic axis in T-cell leukemia.

Telomerase is required for melanoma progression

Joana Nabais, Miguel Godinho Ferreira

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Contrary to other cancers, metastatic melanoma remains practically incurable and is responsible for 90% of skin cancer deaths. There are many genetic alterations associated with melanoma; however, events leading to melanomagenesis remain elusive. Zebrafish models have shown that mutated BRAF or NRAS lead to nevi formation but, similar to the human disease, melanoma progression requires loss of p53 function, which abrogates cellular senescence.

Telomeres, the protective ends of chromosomes, provide a crucial link between cell proliferation and senescence. Since cancer cells require telomerase, a reverse transcriptase responsible for telomere synthesis, for continuous growth, anti-telomerase therapies are currently in clinical trials for various cancers.

Our studies show that telomerase inhibition prevents melanoma. We are currently investigating the requirement of telomerase for melanoma progression. Our data points to a requirement of telomerase at later stages of carcinogenesis including metastasis. We aim to determine the window of opportunity for anti-telomerase therapies in metastatic melanoma by inhibiting telomerase both genetically (using conditional transgenics) and pharmacologically (using anti-telomerase drugs).

0001

Loss of WNK2 expression by promoter gene methylation occurs in adult gliomas and triggers Rac1-mediated tumour cell invasiveness

Sónia Moniz[1], Olga Martinho[2], Filipe Pinto[2], Bárbara Sousa[3], Cláudia Loureiro[1], Maria José Oliveira[3], Joana Paredes[3], Rui Manuel Reis[2], Peter Jordan[1]
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The gene encoding protein kinase WNK2 was recently identified to be silenced by promoter hypermethylation in gliomas and meningiomas, suggesting a tumour suppressor role in these brain tumours. Following experimental depletion in cell lines, WNK2 was further found to control GTP-loading of Rac1, a signalling GTPase involved in cell migration and motility. Here we show that WNK2 promoter methylation also occurs in 17.5% (29/166) of adult gliomas, whereas it is infrequent in its paediatric forms (1.6%; 1/66). Re-expression of WNK2 in glioblastoma cells presenting WNK2 gene silencing reduced cell proliferation *in vitro*, tumour growth *in vivo* and also cell migration and invasion, an effect correlated with reduced activation of Rac1. In contrast, when endogenous WNK2 was depleted from glioblastoma cells with unmethylated WNK2 promoter, changes in cell morphology, an increase in invasion and activation of Rac1 were observed. Together, these results validate the WNK2 gene as a recurrent target for epigenetic silencing in glia-derived brain tumours and provide first mechanistic evidence that the role of WNK2 as a tumour suppressor is related to Rac1 signalling and tumour cell invasion and proliferation.

0002

Androgen-responsive and nonresponsive prostate cancer cells present a distinct glycolytic metabolism profile

Cátia V. Vaz[1], Marco G. Alves[1], Ricardo Marques[1], Paula I. Moreira[2], Pedro F. Oliveira[1], Cláudio J. Maia[1], Sílvia Socorro[1]
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Prostate cancer (PCa) progresses from an early stage, confined to prostate, to a more aggressive metastasized cancer related with loss of androgen responsiveness. Although, it has been recognized that PCa cells have unique metabolic features, their glycolytic profile in androgen-dependent and androgen-independent stages of disease is much less known. Hence, the main purpose of this study was to compare glucose metabolism in androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) PCa cells. Cell culture medium was collected and differences in glucose consumption and, lactate and alanine production were measured using Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra analysis. The mRNA and protein expression of glucose transporters (GLUT1 and GLUT3), Phosphofructokinase 1 (PFK1), lactate dehydrogenase (LDH) and monocarboxylate transporter (MCT4) were determined by real-time PCR and Western Blot, respectively. The obtained results demonstrate that androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) cells consumed similar amounts of glucose, whereas PC3 cells present higher lactate production. This increase in lactate production was concomitant with higher levels of MCT4 protein, increased LDH activity and higher lactate/alanine ratio, also suggesting increased levels of oxidative stress in PC3 cells. However, protein levels of LDH, associated with lactate metabolism, and GLUT3, involved in glucose uptake, were decreased in PC3 comparatively with LNCaP. Androgen-responsive and nonresponsive PCa cells present distinct glycolytic metabolism profiles, which suggest that targeting LDH and MCT4 metabolic pathways may be an important step for the development of new diagnostic and therapeutic strategies in the different stages of PCa.

0003

SARCOPENIC OBESITY IN CANCER: A PRIORITY FOR INDIVIDIALISED NUTRITIONAL INTERVENTION?

Ana Isabel Almeida[1], Carolina Boléo-Tomé[1], Isabel Monteiro-Grillo[1,2], Maria Camilo[1], Paula Ravasco[1]
[1]Unidade de Nutrição e Metabolismo, Instituto de Medicina Molecular e Laboratório de Nutrição, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal, [2]Departamento de Radioterapia, Hospital Universitário de Santa Maria, Lisboa, Portugal

Rationale: In cancer, worldwide data stress the prevalence of cachexia, while growing information draws our attention to overweight/obesity at diagnosis. This pattern may be determined by the patients' diet, which in turn may influence treatment' tolerance and outcome. We aimed to analyse potential associations between relevant nutrients, nutritional status & disease/treatment related symptoms and tolerance. Methods: Cross-sectional study with 426 patients with solid tumours at various stages; weight and height were determined with a Jofre® scale+stadiometer. Body Mass Index (BMI) was calculated and categorised by age/sex reference values. CT scans (L3-L4) were used for body composition analysis. Current intake was assessed by 24-hour recall & usual intake by a 1-year validated food frequency questionnaire (FFQ). Symptoms were assessed by validated/specific Patient Generated-Subjective Global Assessment (PG-SGA). Results: We included 257M:169F; by BMI 4% were underweight vs 64% overweight/obese, and CT scans showed that these patients had a pattern of sarcopenic obesity. The prevalence of symptoms after

adjusting for the sample size, was 85%, more significant in sarcopenic obese patients (p<0.001); sarcopenic obesity was significantly associated with higher priority for nutrition intervention (p<0.005). Conclusion: The majority of patients was overweight/obese with depletion of muscle mass; their diet was characterised by both excessive and insufficient intakes of key nutrients, likely to contribute to nutritional deterioration, toxicity and treatments' tolerance and body composition pattern. Sarcopenic obesity is a highly complex feature of major clinical relevance for patients' prognosis. This emerging clinical scenario urgently argues for randomised clinical trials of nutritional therapy to determine an effective and targeted intervention.

0004

CANCER, DIETARY PATTERN AND SARCOPENIC OBESITY: NEW INSIGHTS

Ana Isabel Almeida[1], Carolina Boléo-Tomé[1], Isabel Monteiro-Grillo[1,2], Maria Camilo[1], Paula Ravasco[1]
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Rationale: Diet is a major risk factor for obesity and for cancer: it may protect from, but can also worsen tissue damage during cancer progression and treatments. This study aimed to characterise the diet pattern of a cohort of cancer patients and to identify excessive and/or insufficient intake of key nutrients. Methods: Cross-sectional study conducted in 426 patients with solid tumours in various stages referred for Radiotherapy; weight and height were determined with a Jofre® floor scale+stadiometer. BMI was evaluated and further categorised by age/sex reference values. Current diet intake was assessed by 24-hour recall and usual diet by a validated 1-year food frequency questionnaire. Food data were analysed by DIETPLAN® to obtain the detailed daily nutrient intake. Results: We included 257M:169F with cancers of the breast, prostate, lung, colon-rectum, head-neck, oesophagus, stomach. Overweight/obesity was prevalent (64%); 85% of pts had an inadequate diet with excessive energy intake, of which lipids represented 39%, with 18% of saturated fat. Additionally, a high intake (>2X DRI) of protein, refined carbohydrates, cholesterol, iron & sodium was found, concomitantly with insufficient intake (<50% DRI) of fibre, folate & vitamins A, D, E, C, especially in breast, lung, prostate & head-neck cancers. Higher BMI was significantly correlated with an inadequate diet (p<0.002). Conclusion: There was a significant, striking and clinically worrying prevalence of inadequate intake of key nutrients during anti-neoplastic treatment(s), in addition to overweight/obesity. It is essential to provide nutritional counseling aiming to prescribe therapeutic diets, with anti-inflammatory, antioxidant and immunomodulatory effects, that may protect from radiation and citotoxic injury. Plus, an adequate intake may modulate body composition that will in turn contribute to improve treatments' tolerance and disease prognosis.

0005

BIOELECTRICAL IMPEDANCE AND PHASE ANGLE: HOW RELEVANT IN CANCER?

Ana Isabel Almeida[1], Catarina Ferreira[1], Isabel Monteiro-Grillo[1,2], Maria Camilo[1], Paula Ravasco[1]
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Rationale: Body composition may be determinant for cancer progression and treatments. This pilot longitudinal study in cancer, aimed to characterise body composition, concomitantly with phase angle (PA); we also explored potential associations with cancer variables: histological aggressiveness and stage. Methods: We included 26 patients with solid tumours at various stages. Height and weight were determined with a Seca® scale+stadiometer. BMI was calculated and categorised according to WHO's criteria; %body fat mass (%FM) and phase angle (PA) were assessed by tetrapolar multifrequency bioelectrical impedance (Biodynamics 450®, Seattle, USA); %FM and PA were compared with age/sex reference values: percentage intervals & percentiles, respectively. Descriptive analysis was performed. Results: Stages III/IV and moderately/poorly differentiated cancers were prevalent: 54% & 69%, respectively. By BMI, 47% patients were overweight/obese vs 6% underweight. Excessive FM was prevalent (65%) and also found in patients with normal BMI. Overall, 29% of patients had PA<5th percentile. The prevalence of stage III/IV and moderately/poorly differentiated cancers was similar in normal BMI/FM as it was in obesity/high FM. In what concerns PA, 86% patients with PA<5th percentile, had cancers of stages III/IV and moderate/low differentiation vs patients with PA>5th percentile, of which 41% & 63% had advanced and more aggressive cancers, respectively. Conclusion: Excessive adiposity by BIA was prevalent and underestimated by BMI. Advanced stage and more aggressive cancers, indicators of worse disease status, were significantly related with a lower PA. Thus, body composition analysis complemented with PA determination, both simple and quick for routine use, seem to bear a high clinical relevance. These preliminary results do support the continuation of this longitudinal, with additional in depth & validation analyses, as well as to study targeted nutritional interventions.

0006

BIOELECTRICAL IMPEDANCE PHASE ANGLE: PREDICTOR OF CELL DISTURBANCE, METABOLISM AND PRIORITY FOR NUTRITIONAL INTERVENTION?

Ana Isabel Almeida[1], Catarina Ferreira[1], Isabel Monteiro-Grillo[1,2], Maria Camilo[1], Paula Ravasco[1]
[1]Unidade de Nutrição e Metabolismo, Instituto de Medicina Molecular e Laboratório de Nutrição, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal, [2]Departamento de Radioterapia,, Lisboa, Portugal

Rationale: Cancer and undernutrition may result in disturbed electric tissue properties, translated in altered phase angle (PA). This pilot study aimed to assess the predictive value of bioelectrical impedance PA in identifying cancer patients with major priority for nutritional intervention. Methods: We included 26 ambulatory pts with different cancers and stages. Nutritional assessment was performed with the validated Patient-Generated Subjective Global Assessment (PG-SGA). PA values were assessed by tetrapolar multifrequency bioelectrical impedance (Biodynamics 450®) and compared with age/sex reference percentiles. PG-SGA scores were expressed as median (interquartile range); comparisons were made using non-parametric tests. Results: Undernutrition was found in 38% of pts, and 71% had indication for urgent nutritional intervention. PA<5th percentile was prevalent in undernourished pts and with indication for urgent nutritional intervention (44%). Median PG-SGA intervention score was significantly higher in pts with a PA<5th percentile vs a PA>5th percentile [12.5 (8.5-17.5) vs 4 (2-7), p=0.005]. Median PG-SGA scores on food intake, symptoms & functional capacity were worse in pts with a PA<5th percentile vs patients with PA>5th percentile (p<0.05). Overall, PA<5th percentile did predict a significantly worse PG-SGA score (p=0.005). No significant differences were found on PG-SGA B, C & D scores. Conclusion: A PA<5th percentile was associated with critical need for nutrition intervention. PA integration in clinical practice may be of great value; while simple and easy to use, it provides key information on cell disturbance and metabolism; this information may be useful as a first approach to prioritize the critical need of nutritional intervention and symptom management.

0007

BIA PHASE ANGLE IN CANCER PREDICTS QUALITY OF LIFE AND PROGNOSIS

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Rationale: Bioelectrical phase angle (PA) in cancer has been suggested as a potential prognostic disease indicator, and as a consequence is likely to predict patients' well-being and Quality of Life (QoL), a gold standard for any clinical intervention. This pilot longitudinal study aimed to assess the value of PA in predicting cancer patients' QoL. Methods: 26 patients with different cancers and stages, referred for Radiotherapy were evaluated. PA was assessed by tetrapolar multifrequency bioelectrical impedance (Biodynamics 450®, Seattle, USA) and compared with age/sex reference percentiles. QoL was assessed by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire version 3.0. QoL scores were expressed as median(interquartile range); comparisons were made using non-parametric tests. Results: PA<5th percentile was found in 29% of patients. Median global QoL & self-rated health status (SRHS) scores were similar [71 (57-71) and 71 (57-82), respectively]. Yet, poorer SRHS was associated with PA<5th percentile [50 (43-63) vs 71 (57-86), p<0.05]; no statistically significant difference was found for global QoL score [64 (50-71) vs 71 (57-86), NS]. Furthermore, physical, role, emotional and social functions were significantly impaired in pts with PA<5th percentile (p<0.05). On symptoms scales, fatigue, nausea/vomiting, insomnia & anorexia were worse in patients with PA<5th percentile (p<0.05). Conclusions: PA<5th percentile did predict poorer SRHS & anticipated impaired functional scores, as well as worse symptoms. Thus, at diagnosis and/or disease onset, the 5th percentile for PA cut-off, may allow the identification of patients with worse QoL dimensions, SRHS and symptoms, factors associated with poorer nutritional status and intake, all proven to be modulated & improved by individualised nutritional intervention, thus corroborating the need for adjuvant nutrition as therapy.

0008

N-acetylglucosaminyltransferases III and V regulate E-cadherin stability at the cell membrane. Implications in the Epithelial to Mesenchymal Transition.

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E-cadherin is a cell-cell adhesion molecule whose dysfunction or inactivation is a common feature of invasive carcinomas. In addition, E-cadherin is a well-accepted marker of phenotypic plasticity, and is a central

molecule during Epithelial to Mesenchymal Transition (EMT) signaling pathway, that occurs during embryonic development, tissue regeneration, and thought to occur in cancer initiation/progression. The post-translational regulation by N-glycosylation through GnT-III and GnT-V glycosyltransferases has been reported by others and us to be an alternative mechanism of E-cadherin functional regulation. We have demonstrated that GnT-III induced a stabilizing effect on E-cadherin at the cell membrane by inducing a delay in the turnover rate of the protein which promotes the formation of stable and functional adherens-junction, and further prevents clathrin-dependent E-cadherin endocytosis. This contributes to E-cadherin-mediated tumor invasion suppression function. Conversely, GnT-V promotes the destabilization of E-cadherin, leading to its mislocalization together with formation of unstable adherens-junctions and impairment of cell-cell adhesion, therefore contributing to tumor progression. This stabilizer/destabilizer effect of GnT-III and GnT-V on E-cadherin was further validated in clinical samples of human invasive carcinomas. Furthermore, we also found that during Epithelial to Mesenchymal Transition (EMT), Mgat3 expression was dramatically decreased and later recovered when cells returned to an epithelial-like phenotype (Mesenchymal to Epithelial Transition (MET)). We further identified that Mgat3 promoter methylation/demethylation is a mechanism involved in this expression regulation. The impact of Mgat3/GnT-III expression variation, along EMT/MET, was accompanied with a specific modification of E-cadherin glycosylation with bisecting GlcNAc structures. These results open new insights into the molecular mechanisms associated with the regulation of E-cadherin in tumor cells with potential translational clinical and therapeutic applications. In addition, we have identified for the first time Mgat3 glycogene expression and GnT-III-mediated glycosylation, specifically on E-cadherin, as a novel and major component of the EMT/MET mechanism signature, supporting its role during EMT/MET.

0009

Role of CDX2 on the regulation of the cancer-associated Sialyl-Tn carbohydrate antigen

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De novo expression of sialyl-Tn antigen is one of the most common features of gastric intestinal metaplasia (IM) and gastric carcinomas (GC). However, the regulation of its expression is not fully elucidated. Previous studies identified the homeobox transcription factor CDX2 as a direct regulator of MUC2 mucin expression, the major carrier of sialyl-Tn in IM and GC. We therefore hypothesized that CDX2 might induce a cancer-associated glycoproteome alteration in the gastric context - MUC2-sialyl-Tn - by concomitant regulation of *ST6GalNAc-I*, which encodes the sialyltransferase responsible for STn biosynthesis, and *MUC2* genes. In this study, our aim is to evaluate whether CDX2 transactivates *ST6GalNAc-I* in a gastrointestinal model, both *in vitro* and *in vivo*. The *in vitro* spontaneous differentiation of Caco-2 cells induces an increase in CDX2 expression which is accompanied by concomitant increase in *ST6GalNAc-I* expression. On the other hand, CDX2 silencing using siRNAs in AGS cells was followed by a decrease in *ST6GalNAc-I* transcriptional levels. To clarify the mechanisms underlying *ST6GalNAc-I* gene transcriptional regulation by CDX2, luciferase assays were performed. Co-transfection of gastric and colonic cell lines with pGL3-derived constructs covering 1.6kb of the human *ST6GalNAc-I* promoter and a CDX2 expression vector confirmed a transactivation of *ST6GalNAc-I* promoter. Moreover, chromatin immunoprecipitation (ChIP) was carried out in order to identify relevant CDX2-binding regions to the *ST6GalNAc-I* promoter. ChIP analysis proved that CDX2 was bound to it. Our work supports the novel concept that a single homeobox gene, CDX2, is orchestrating a glycoproteome modification during cancer development. Future perspectives include validation of the results by site-directed mutagenesis of the *ST6GalNAc-I* promoter, EMSA and the use of a Proximity Ligation approach for *in vitro* and *in vivo* detection of DNA-protein interactions. Moreover, *in vivo* ChIP will be performed using human gastric IM and cancer samples to validate our hypothesis on gastric carcinogenesis.

0010

A Novel MUC16 (CA125) Monoclonal Antibody

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Introduction: The MUC16 mucin was identified as the serum antigen detected in the CA125 biomarker assay used to monitor patients with ovarian cancer. A number of monoclonal antibodies (MAbs) including OC125 and M11 are available to detect MUC16. Despite considerable efforts the epitopes detected by these MAbs have remained elusive and glycosylation has been proposed to play a role for the epitopes. Existing MAbs react with MUC16 expressed in both normal cells and in cancer and hence detect enhanced levels of MUC16 derived from both benign and malignant cells.

Material and Methods: In this study, we used an *E.coli* expressed MUC16 fragment glycosylated in vitro with Tn for immunization of mice and selected a novel MAb

(5E11) with a cancer reactivity. Overlapping peptides covering the tandem repeat domain were used for epitope mapping in ELISA and microarrays assays.

Results and Discussion: Comprehensive analysis of the fine specificities of existing MUC16 MAbs and the new MAb have been undertaken and we found that existing MAbs react with a conformational epitope of the 156 amino acid tandem repeat sequence of MUC16 without dependence of O-glycosylation. The novel MAb in contrast reacts with a linear peptide epitope, which expression is dependent on glycosylation.

Conclusion: We characterized existing MAbs for MUC16 (M11 and OC125) and produced a new MUC16 MAb (5E11) that recognizes a glycosylation dependent epitope on the tandem repeat region of MUC16.

0011

The social network: testing the communication between tumor cells and the surrounding stroma in tumor development

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It is now generally accepted that a tumor is a heterogeneous entity composed of a wide range of cell populations in different stages of differentiation. Under this assumption, a specific population of cells with stem-like properties have been searched for and successfully isolated from tumors of different origins. These cancer stem cells (CSCs) were later implicated in tumor aggressiveness and resistance to conventional therapies as well as tumor relapse.
Aiming to study the molecular mechanisms underlying hexavalent chromium [Cr(VI)] induced lung cancers, we malignantly transformed the normal human bronchial epithelial cell line BEAS-2B into the RenG2 system, using low density culture in the presence of Cr(VI). Two additional cell lines (DRenG2 and DDRenG2) were attained following serial rounds of injection in nude mice. Characterization results allowed us to identify different cellular sub-populations within each cell line, and prompted the hypothesis that CSCs may have driven BEAS-2B' malignization. Sphere-formation assay was used to search and isolate these cells, which were only present in DRenG2 and DDRenG2 cell lines, forming more and bigger spheres in the DDRenG2. This suggested that a dedifferentiation process featured the formation of CSCS during RenG2 derivation in nude mice. To access the involvement of mice stroma in this process, chirurgical-isolated mouse stromal cells of the subcutaneous compartment were co-cultured with RenG2 cells for 30-60 days (time needed to induce tumors in mice with RenG2), which resulted in the emergence of a CSCs sub-population. We are now able to attest that CSCs may emerge in a tumor as a consequence of stromal-emitted paracrine signals, which brings deep implications to future therapeutic approaches.

0012

On the origin of cancer stem cells

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Tumors are characterized by their cellular heterogeneity due to the co-existence of different cellular sub-populations, whose hierarchic organization in certain cancers lead to the hypothesis that the target cells of transforming mutations are stem cells. However, in other tumors, restricted progenitors or even differentiated cells may be the cell of origin. Cancer stem cells (CSCs) are consequently, stem-like cells with self-renewal and multipotent differentiation characteristics which can originate all cell types found in a tumor (1).Unexpectedly, while attempting to understand the mechanisms underlying hexavalent chromium induced lung cancer, we demonstrated that CSCs could be obtained by dedifferentiation of the malignant bronchial epithelial cells DRenG2 and DDRenG2 and/or their precursor RenG2. The present work evaluated the proliferation rate of Cont-1, RenG2, DRenG2 and DDRenG2, and their normal precursor BEAS-2B cells, the cytogenetic evolution from BEAS-2B to DDRenG2, the epithelial/mesenchymal phenotype of the cell lines. Finally, the chemoresistance to gemcitabine and cisplatin, was evaluated and correlated to the presence of the efflux pump P-Glycoprotein (P-gp). The cytogenetic analysis of the more malignant and more proliferative DRenG2 and DDRenG2 cell lines revealed a common structural difference relative to progenitor BEAS-2B cells namely $7p^-$. However, DRenG2 revealed the predominance of $7q^-$ and $iso9q^+$ while DDRenG2 t(7:14) and $17q^+$. In contrast to the less proliferative BEAS-2B, and similarly to RenG2 both DRenG2 and DDRenG2 predominant ploidy was 75/76 chromosomes. Immunocytochemistry analysis revealed that all cell lines were mesenchymal –like cells (MNF116- and Vimentin-positive). As expected the more malignant cell lines were significantly more resistant to gemcitabine (GEM) and cisplatin (cDDP). Although, quite often multidrug resistance is associated to the overexpression of the membrane efflux pump P-gp, other mechanism(s) may account for the observed resistance of DRenG2 and DDRenG2 cells as all the cell lines were P-gp negative.

0013

SENESCENT BRONCHIAL FIBROBLASTS DRIVE BRONCHIAL EPITHELIAL CELLS METAPLASIC TRANSFORMATION FOLLOWING EXPOSURE TO HEXAVALENT CHROMIUM

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Cellular senescence, a phenomenon associated with aging and/or stress insults, can prevent neoplastic transformation. Nevertheless, accumulated evidence revealed that senescent cells can stimulate malignant phenotypes in nearby cells by secreting protumorigenic factors that stimulate epithelial cells proliferation and disrupt epithelial differentiation. Senescent stromal cells also stimulate the acquisition of invasive and migratory phenotypes by promoting epithelial to mesenchimal transition (EMT).
Here we report that sub-cytotoxic doses (0.25 and 0.5 µM) of hexavalent chromium [Cr(VI)], a human lung carcinogen known to induce squamous cell carcinomas, induced the senescence of normal human bronchial fibroblasts (E2A) which stimulated the proliferation and a striking change in the phenotype of human bronchial epithelial cells (BEAS-2B). In fact, co-cultivating senescent E2A with BEAS-2B cells in presence of 0.25 µM Cr(VI) stimulated BEAS-2B cells to acquire a basal cell phenotype (MNF+, Vimentin+) with large round nuclei and tadpole cytoplasm characteristic of epidermoid metaplasia. Furthermore, co-cultures exposed to 0,5 µM Cr(VI) lead BEAS-2B cells to acquire a cuboid morphology with many mitotic figures and activated nuclei with visible nucleoli, as well as fusiform shape associated with EMT phenotypic switch. Additionally, senescent E2A fibroblasts co-cultured with Cr(VI)-treated BEAS-2B cells acquired mesenchymal features i.e., Vimentin+ and α-SMA+ large stellate cells with heterogeneous size enlarged nuclei, particularly abundant for 0.5 µM Cr(VI) which also lead to the appearance of crisscrossing. Altogether our results suggest that in presence of Cr(VI) the crosstalk between senescent fibroblasts and epithelial cells, mediated by secreted factors by both cell types, induced premalignant characteristics in BEAS-2B cells and a more undifferentiated phenotype in fibroblasts.

0014

The Early Growth Response Genes as therapeutic targets in colon cancer

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The Egr family of zinc finger transcription factors, which consists of four members; Egr-1, -2, -3 and -4, has dynamic functions in the regulation of cell growth, developmental biology and immune responses. However, their roles in the development of tumour are not clear. In this study, we investigated the function of three closely related Egr family members, Egr-1, -2 and -3 in the regulation of growth of colorectal cancer cells. Two cell lines deriving from human colon cancer; one p53 negative (DLD1) and another p53 positive (HCT116) were transfected with Egr-1, -2 and -3, respectively. We found that all three Egr members can suppress tumour cell growth suggesting that the function of Egr in the control of cell growth is not associated with the function of p53. In addition to the growth arrest, the transfected cells changed morphology to round shape indicating of senescence. This may suggest that Egr molecules are important to control the unwanted growth in response to malignant transformation. Our results not only demonstrated an important function of Egr molecules and also indicate the therapeutic potential for the treatment of tumour.

0015

Evaluation of the role of immunohistochemistry markers in the differential diagnosis of adrenocortical tumors

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Malign adrenocortical tumors are rare and highly aggressive, conversely benign tumors are more common and frequently found incidentally. The diagnosis of these tumors is based only on histological characteristics, since there are no established molecular markers.

The aim of the present study was to analyze the molecular profile of different adrenocortical tumors with the purpose of identifying useful markers for the differential diagnosis.

The adrenocortical tumors studied (n=31) were, non-functioning adenomas/incidentalomas (n=13), functioning adenomas with Cushing syndrome (n=7),and carcinomas (n=11); Normal adrenal glands (n=12) were used as controls. For each sample, the percentage of the stained area and the QIC score (Quantitative

immunocitochemical score) by immunohistochemistry were quantified for StAR, IGF2, p53, Mdm2, p21, p27, cyclin D1, Ki-67, β-catenin and E-cadherin, using a morphometric computerized analysis tool.

Of the studied markers, IGF2, p27, cyclin D1 and Ki-67 were those whose percentage of marked area and QIC score was significantly higher on carcinomas when compared with all the adenomas. Comparing the carcinomas with the functioning Cushing syndrome adenomas, we observed significant differences in the percentage of the stained area and QIC score for p27 and Ki-67, which was increased and for StAR that was decreased in carcinomas. Comparing the carcinomas with incidentalomas, the marked area for IGF2, p27, cyclin D1 and Ki-67 was significantly higher on carcinomas. The p27 and the Ki-67 were the markers that showed the highest discriminative power for the differential diagnosis between carcinomas and adenomas, while the IGF2 and StAR only demonstrated to be useful for the differential diagnosis between carcinomas vs non-functioning adenomas and carcinomas vs adenomas with Cushing syndrome, respectively.

The use of the markers StAR, IGF2, p27, cyclin D1 and Ki-67, and the quantification of its expression by computerized morphometric analysis could be an important auxiliary means on the differential diagnosis of adrenocortical tumors.

0016

Centrosome abnormalities in Barrett's malignant transformation

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Barrett's esophagus (BE) is a clinically important premalignant condition that develops in the context of chronic gastroesophageal reflux disease. In BE the normal squamous epithelium is replaced by a metaplastic columnar lining with goblet cells. It is a multistep process from metaplasia to dysplasia and carcinoma that is accompanied by a progressive accumulation of well-characterized genetic lesions that are observed in many other solid tumours. BE is the only known precursor of esophageal adenocarcinoma, a tumour whose incidence has increased profoundly over the last decades. Yet, only 0.12% of individuals with BE develop esophageal adenocarcinoma per year and, to this date, besides dysplasia there are no clinical or morphological markers to identify the patients that will progress. BE and associated neoplasia exhibit abnormalities on cellular processes controlled by the centrosome, the primary microtubule-organizing centre in animal cells. Several studies have shown that cells from many cancers have abnormal centrosomes that are either correlated with tumour malignancy or considered an early event during tumorigenesis. However, a causative link between centrosome abnormalities and cancer remains elusive. In this study, we set out to investigate how centrosome defects contribute to tumorigenesis using BE as a model. Centrosome and centriole profile were assessed in paraffin-embedded BE biopsies and esophagectomy specimens of Barrett's adenocarcinoma as well as in established cell lines derived from human esophageal adenocarcinoma. Using immunofluorescence and electron microscopy we found that both numerical and size centriole defects progressively accumulate in BE tumorigenesis. These findings suggest that centrosome abnormalities are related to Barrett's tumour progression and may even be involved in its early steps. This study brings us closer to a better understanding of how centrosomal abnormalities relate to genetic changes observed in BE, and may provide new opportunities for advances on patient management strategy for early detection and prevention.

0017

Germinative cell tumors support bronchial-pulmonary development through cell lineages:Immunohistochemical study

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The purpose of this study was to stratify the morphology of germ cell tumors (GCT) to correlate embryogenesis, organogenesis and tissue maturation with concordant immunohistochemical antibodies. Several studies support the idea that carcionogenesis carries on cellular differentiation and this theory was explored in germinal cell tumors to understand cell lineage in neoplastic development that would establish their classification according with a specific imunohistochemical panel to identify pluripotent stem cells and adult stem cells.

The antibodies AE1/AE3, CK7 and LP34 (cellular maturation), CDX2, TTF1 and PLAP (organogenesis) and Oct3/4, Nanog and Vimentin (embryogenesis) were applied to 34 benign and malignant FFPE GCT, concerning 17 males and 17 females (age range 16-73 years).The immunohistochemical results were scored semi-quantitatively by the percentage of positive cells in a scale of 0% (0), <10% (1+),10-50%(2++) and > 50%(3+++) considering cytoplasm or nuclei expression.

Our results showed Oct3/4 expression in nuclei of seminoma cells while embrionary carcinoma cells expressed either nuclear and cytoplasm positivity; Nanog gene expression was seen only in 2 cases of less differentiated tumors. Vimentin came out as a particular antibody in between Oct3/4 expression and CDX2/TTF1 cellular maturation, indicating its value in the transition between organogenesis and adult stem cells maturation. Cellular maturation was seen in neoplasias with intestinal differentiation due to CDX2 expression, TTF1 was sensitive for pulmonary alveolar epithelium. PLAP glycoprotein showed positive expression in the majority of embryonic carcinomas.Cytokeratin AE1/AE3 was not discriminatory because it was expressed in all cases and corresponded to a non-specific epithelial marker.

We may stress that the cellular maturation spectrum seen since embryogenesis till adult stem cells in GCT is similar to malignant cell lineage development in carcinogenesis, according with the actual grades of differentiation, also validating epithelial-mesenchimal transition observed in less differentiated tumors/carcinomas at least of some organs, including bronchial-pulmonary carcinomas.

0019

Ncf1-deficient mice with impaired oxidative bursthave a more aggressive progression of Dextran Sulfate Sodium (DSS) -induced colitis

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Introduction

The most common clinical patterns in IID is chronic colitis and epithelial dysplasia. Intestinal Inflammatory Disease (IID) as a primary immunodeficiency depends on mutations in the NADPH oxidase complex, responsible for the production of reactive oxygen species (ROS). Ncf1-mutation in mice leads to deficiency in ROS, rendering them susceptible to autoimmunity. Here we studied how ROS-deficiency of reactive oxygen species (ROS) in Ncf1-mutant mice influenced epithelial dysplasia.

Material and Methods

Colitis was induced in wild type (WT) and Ncf1-mutant (Ncf1) B10.Q mice by administration of 3.5% DSS in the drinking water for one week. After one week recovery, DSS was administered for another week. Mice were sacrificed at days 0, 7, 14 and 21, the colon was removed and folded into a Swiss roll. Sections of the colon were stained with HE and dysplasia was considered either in wild-type and Ncf1-mutant mice.

Results/ Conclusions

Epithelial dysplasia was high grade in Ncf1-mice together with poor epithelium recovery (hyaline scars). The atypical cells with mitotic higher rate were clearly located in the bottom of the colonic crypts. As ROS appeared to be crucial for leukocyte recruitment and tissue-repair in DSS-induced colitis,it was seen that re-epithelization over inflammatory cells isprone to develop dysplasia (apparently high grade) *ab initio*. These results are usefull to understand the need of continuous anti-inflammatory treatment of ID patients as earlier as possible.

0020

Bronchial-Pulmonary Adenocarcinomas Subtyping by PET Scanning

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Introduction:

Bronchial-pulmonary carcinomas have 5 year survival poor, between 6% and 14% in men and 7% to 18% in women. Treatment depends on clinical staging and morphological classification made in biopsies concerning 70% of the cases. This is the actual state after all therapeutic and diagnosis effort.

Aim:

Immunohistochemical expression between different histological types was compared with Max 18F-Fluordesoxiglucose as the clinical parameter based in PET, to preview diagnosis and prognosis.

Methods:

The immunohistochemistry study was performed in 41 surgical specimens including Adenocarcinomas (18), Epidermoid Carcinomas (12) and the heterogeneous groups of Large Cell Neuroendocrine Carcinoma (3), Small Cell Lung Cancer (1) , Large Cell Carcinoma (2), Adenosquamous Carcinoma (2) and Pleomorphic Carcinomas (3) Max 18F-Fluordesoxiglucose (FDG) was the clinical parameter applied to validate the Pathological Subtyping.

Results:

Significant differences (p=0.006) between TTF-1 positive and negative Adenocarcinomas where translated as 18F-FDG capture was lower in TTF-1 positive cases, indicating lower metabolic activity. Epidermoid Carcinomas and TTF-1 negative Adenocarcinomas have similar and higher metabolic activity. The other histological types have FDG capture similar and in between the two defined groups.

Conclusion:

The clinical differences between Adenocarcinomas and Epidermoid Carcinomas related with

immunohistochemical expression of TTF1 and 18F-FDG capture showed TTF-1 negative Adenocarcinomas as biologically similar to Epidermoid Carcinomas needing a different medical approach as well as molecular pathology particular interpretation. These results clearly raise need of recognizing TTF-1 negative Adenocarcinomas because they are different from the terminal respiratory unit TTF-1 positive Adenocarcinomas.

0021

IL-6-174 as a Gastric Carcinogenesis Marker in Biopsies

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Introduction
Gastric carcinoma is related with cancer genetic susceptibility that can be investigated through single nucleotide polymorphisms (SNPs) and as cytokine genes are known to predispose to malignant disease, several polymorphisms of Interleukin-6 (IL-6) gene have been reported to be associated with tumour progression including inhibition of malignant epithelial cells apoptosis and stimulation of angiogenesis.

The aim of this study was to understand the association between IL-6 polymorphisms and the risk for gastric cancer and chronic gastritis maintenance.

Materials and Methods

PCR-SSP genotyping for IL-6 -174C>G polymorphism was performed in 100 biopsies of gastric carcinoma and in 100 biopsies of chronic gastritis.

Results
There was association between IL-6 -174C allele (p=0,0466) and -174CC, low producer, genotype (p=0,0466) and gastric carcinoma, whereas IL-6G allele (p=0,0278) and IL-6GG (p<0,0001), high producer, genotype was associated with gastritis.

Conclusion
We conclude that IL-6 -174, low producer genotypes, may have an important role in gastric carcinogenesis and the polymorphism study of this molecule could be a good marker for gastric carcinoma susceptibility when high grade dysplasia is seen in biopsies.

0022

ALK Mutation Detection in Bronchial-Pulmonary Adenocarcinomas for Tirosine-kinase Inhibition Prescription

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Introduction
Low cost of validation of EML4-ALK for crizotinib therapy and a rapid answer in Pathology routine was searched in a series of 35 bronchial-pulmonary carcinomas: 20 adenocarcinomas, 6 epidermoid carcinomas, 4 pleomorphic carcinomas (mixed type adenocarcinomas with large/giant/fusiform cells), 4 neuroendocrine carcinomas (NEC 1 combined large cell NEC with adenocarcinoma and 2 with combined epidermoid carcinomas; 1 SCLC chromogranin positive combined with adenocarcinoma) and 1 adenosquamous carcinoma were selected.

Materials and Methods
The criteria of Histological/WHO 2004 classification and CK7, TTF1, CK5.6, CD56/chromogranin, vimentin and ALK (clone 5A4, Novocastra Laboratories Ltd, Newcastle, United Kingdom) immunohistochemical (IHC) panel were applied, in addition the commercially available LSI ALK Dual Color, Break Apart Rearrangement Probe set (Vysis, Abbott) was used on all specimens to detect ALK-rearrangements by fluorescence in situ hybridization (FISH).

Results
The IHC panel specified bronchial-pulmonary carcinomas subtypes clearly. In 3 cases, ALK expression had over 50% expression in malignant cells: mixed type adenocarcinomas with acinar, solid, micropapillary and microacinar patterns; one glandular mucinous pattern (mucinous BA pattern) and one BA pattern, all expressing TTF-1, corresponding to 3 non-smoking women, over 60 years old. These tree cases were also ALK- FISH positive.

Conclusion
In this study, where 3/20 adenocarcinomas of older women had ALK protein expression, only one with a mucinous pattern, had also FISH fusion gene detected. EML4-ALK represents a unique subset of bronchial-pulmonary adenocarcinomas and the challenge remains to incorporate and disseminate widespread use of diagnostic testing for an effective therapeutic strategy. Described by S. Lantuejoul, it seems reasonable to apply FISH as the most appropriate method under the point of

view of economical purpose. It is now necessary to decide whether *KRAS* and *EGFR* mutations have to be determined together and/or select TTF-1 positive adenocarcinomas (from terminal respiratory unit) as raised by this approach.

0023

The two stemness-associated genes TAZ (WWTR1) and CYR61 are early markers of Barrett's esophagus malignant progression

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Barrett's esophagus (BE) is the major risk factor for esophageal adenocarcinoma (EA). Given the low but non-neglectable risk of BE progression to EA and the costs associated with monitoring such progression it is imperative to identify new risk stratification markers. Our aim was to look for potential very early biomarkers of BE malignant progression.
We analyzed three publicly available microarray datasets with an innovative bioinformatics biomarker prioritization pipeline that included Gene Expression Barcode 2.0 and differential expression analysis. Candidate genes were validated by qRT-PCR and immunohistochemistry (IHC) in formalin fixed paraffin embedded samples from BE patients with high-grade dysplasia/EA diagnosed during surveillance (EA-progressed) and of their index endoscopy dysplasia-free samples. As controls, we used samples from BE patients who have not progressed (EA-free).
Under conservative criteria, we identified 19 up-regulated genes that distinguish EA-progressed from EA-free BE samples. A second filter, followed by qRT-PCR validation, trimmed the candidates to the two markers TAZ (WWTR1) and CYR61, two genes previously implicated in malignancy-associated epithelial-to-mesenchymal-transition (EMT) and stemness phenotypes. Importantly, qRT-PCR on time-series BE index samples showed that these genes are up-regulated years before the development of EA in EA-progressed as compared to patients who remain EA-free. IHC for the selected markers corroborated the qRT-PCR results. Finally, over-expression of TWIST1 and focal down-regulation of E-cadherin, two known EMT markers were verified by qRT-PCR and IHC, respectively in the EA-progressed versus EA-free BE index biopsies.
The two EMT-related genes TAZ and CYR61 were identified as early risk markers for BE-associated neoplasia. Both genes have a potential role in BE risk stratification and their up-regulation, along with TWIST1/E-cadherin changes suggests the involvement of EMT/stemness properties in the very early stages of BE malignancy. Plus, our innovative bioinformatics pipeline may be generalized to other tumors/diseases.

0024

Glycoproteomic analysis of serum from patients with gastric precancerous lesions

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Gastric cancer is preceded by a carcinogenesis pathway which includes gastritis caused by *Helicobacter pylori* infection, chronic atrophic gastritis that may progress to intestinal metaplasia (IM), dysplasia and ultimately gastric carcinoma of the more common intestinal subtype. The identification of glycan changes in the precursor lesions of gastric cancer is of high interest and could be used as a source for finding new biomarkers for early diagnosis applications [1]. This study applies a glycoproteomic approach to identify glycoproteins expressing simple mucin-type carbohydrate antigens T and STn in the serum of patients with gastritis, IM (complete and incomplete sub-types) and in control healthy individuals. The immunohistochemistry analysis of the gastric mucosa of these patients showed expression of T and STn antigens in gastric lesions, with STn being expressed only in IM. In order to identify novel serum biomarkers associated with gastric cancer development the following methodology was performed: equalization of serum protein content using combinatorial peptide ligand libraries followed by protein separation by 2D gel electrophoresis; the glycoproteins carrying truncated glycans were detected by 2D Western blot using monoclonal antibodies against these glycan antigens and identified by MALDI-TOF/TOF mass spectrometry. Structural characterization of the candidate biomarkers including glycosylation site assignment at peptide / aminoacidic level and glycoform composition determination is currently being performed. Some of the identified glycoproteins are promising since they have been reported in *H. pylori* chronic infection of the gastric mucosa and in gastric cancer cell invasion.

[1] Reis CA, Osorio H, Silva L, Gomes C, David L. Alterations in glycosylation as biomarkers for cancer detection. J Clin Pathol. 2010; 63:322-9.

0025

THE CO-LOCALIZATION OF CARCINOMAS AND ADENOMAS FAVORS A REGIONAL FIELD DEFECT IN THE COLON

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INTRODUCTION: The finding of common genetic alterations in colorectal cancers (CRC) and flat peri-tumoral mucosa, in ulcerative colitis and, recently, in sporadic cancer led to the notion of colonic mosaicism. The proposed explanations remain controversial and one of them relates to common origins from embryonic stem cells.

AIMS & METHODS: The authors aimed to explore colonic mosaicism by correlating CRC location with the presence and location of synchronous adenomas. All patients submitted to surgery for CRC between November 2006 and June 2010 who had a total colonoscopy performed in the same institution in the 2 peri-operative years were included. Patients' sex and age, tumor and adenomas' location and the presence of adenomas larger than 1cm, with villous component or high grade dysplasia were recorded. Statistics: T test, Chi-square, Exact, Logistic regression (SPSS18).

RESULTS: 199 CRC patients included (57% male, mean age 67,4 years), 89 (45%) of them with synchronous adenomas. The presence of synchronous adenomas was independent of the CRC location (p=0,60). When rectal cancers were excluded, there was a significant correlation between the location of the CRC and the location of all adenomas (p=0,03) and adenomas larger than 1cm (p=0,01). Adenomas of the right colon were more frequent in patients with right colon CRC (p=0,01) and the same happened on the left colon (p=0,01). The presence of adenomas in the right colon was influenced by gender, total number of adenomas and CRC's location (p=0,03/0,01/0,045), while the presence of adenomas in the left colon correlated only with the CRC's location (p=0,02).

CONCLUSION: The correlation between the locations of the CRC and the synchronous adenomas, mostly the larger ones, may derive from the distinct embryonic origins of the right and left colon and point to a common early defect. According to some authors, this association may lead to changes in CRC's surgical approach.

0026

LDL-cholesterol favors leukemia immune evasion through modulation of genes associated with susceptibility and resistance to γδ T cells.

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Cancer development and progression is greatly influenced by the immune system and tumor immune evasion has been recognized as an emerging hallmark of cancer. Among the cells that mediate tumor immune surveillance, γδ cells comprise a distinct subset of T lymphocytes with potent innate anti-tumor activity, in particular against hematological cancers. Leukemic blasts, as well as many other types of malignant cells, have increased needs for many major metabolites, e.g. cholesterol, and we have previously shown that a cholesterol-rich microenvironment favors leukemia engraftment, spread and survival through VEGF signaling.

We performed an Affimetrix Microarray to characterize the molecular alterations, of potential importance for immune responses, which cholesterol induces in B-cell acute lymphoblastic leukemia cells (B-ALL, 697 cell line). Untreated cells were compared with those exposed to low density lipoprotein-cholesterol (LDL) for 12 and 36 hours. Some of the gene expressions that were most dramatically up- or down-regulated by LDL have been implicated in increased susceptibility/resistance of leukemic blasts to γδ T cells. The LDL-mediated modulation of expression of 16 genes, found to govern the interaction between γδ T cells and different hematologic tumor types, was further analyzed by RT-qPCR in a panel of 10 leukemia cell lines. More importantly, B-ALL cells exposed to LDL for 36 hours were protected from cytotoxic killing by activated γδ T cells.

These preliminary data suggest that LDL-cholesterol impairs the interaction between leukemia cells and γδ T cells, favoring tumor cell escape via modulation of genes involved in susceptibility vs resistance.

0027

CDX2 regulation by the RNA-binding protein MEX3A: impact on intestinal differentiation and stemness

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BACKGROUND & AIMS: The homeobox transcription factor CDX2 plays a key role in specifying intestinal cell fate, both in normal development and in tumorigenic processes of the gastrointestinal tract, implying a need for tight regulation. Our objective was to identify new CDX2 regulatory mechanisms that might help to understand the complexity of these processes.

METHODS: Through genome-wide screening of a three-dimensional culture system comprising the gastric carcinoma AGS cell line and an extracellular matrix (Matrigel), we disclosed the RNA-binding protein MEX3A as a putative CDX2 regulator. Biological relevance of this regulation was addressed by modulating MEX3A levels in cell assays, performing RNA-immunoprecipitation and luciferase reporter experiments and assessing its expression in mouse intestine.

RESULTS: We demonstrated that MEX3A exerts a translational repressive role over CDX2 in cellular models of gain- and loss-of-function, both in gastric and colorectal cell lines. Then we proved interaction of MEX3A with *CDX2* mRNA 3`untranslated region and defined the specific binding determinant. We further assessed that it impairs intestinal differentiation and cellular polarity and increases the expression of intestinal stem cell markers, namely Olfm4, Lgr5 and Msi1. Finally, we showed that MEX3A is expressed in mouse intestine, supporting an *in vivo* context for interaction with CDX2 and modulation of stem cell features.

CONCLUSIONS: We have uncovered a novel post-transcriptional regulatory mechanism, through the RNA-binding protein MEX3A, with a major impact in intestinal differentiation, polarity and stemness, likely contributing to gastrointestinal homeostasis and carcinogenesis.

0028

CK2 Activity Regulates Signaling, Survival and Proliferation Mediated by IL-7 in T-Cell Acute Lymphoblastic Leukemia Cells

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Interleukin 7 (IL-7) and its receptor (IL-7R) are essential for normal T-cell development and homeostasis. However, they also decisively contribute to the viability and proliferation of T-cell acute lymphoblastic leukemia (T-ALL) cells. On the other hand, the pleiotropic serine/threonine kinase CK2 is overexpressed and hyperactivated in leukemia, including in T-ALL. CK2 phosphorylates and thereby inactivates PTEN, contributing to constitutive activation of PI3K/Akt pathway. To determine whether CK2 is involved in IL-7-mediated signaling, we treated HPB-ALL (IL-7-responsive) and TAIL7 (IL-7-dependent) cells, with two CK2-specific pharmacological inhibitors: 4,5,6,7-tetrabromobenzotriazole (TBB) and CX-4945, the latter being in phase I clinical trials for several cancers. The efficacy of the inhibitors was first confirmed by assessing the phosphorylation of Akt (S129), a CK2-specific phosphorylation site, or by CK2 in vitro kinase activity assays. Stimulation of T-ALL cell lines with IL-7 had a very minor positive effect on CK2 activity. However, treatment with TBB or CX-4945 revealed that CK2 inhibition completely abrogated IL-7-mediated activation of PI3K/Akt pathway and prevented STAT5 activation. These results suggest that although CK2 activity is not regulated by IL-7, it is fundamental for the activation of two major IL-7-dependent survival pathways. In agreement, both CK2 inhibitors completely prevented IL-7 viability effects not only in T-ALL cell lines but also in primary leukemia cells collected from patients at diagnosis. Accordingly, IL-7-induced Bcl-2 upregulation and maintenance of mitochondrial transmembrane potential were both reversed by treatment with the CK2 antagonists. Furthermore, CK2 inhibition completely abrogated T-ALL cell proliferation. In summary, our study contributes to the elucidation of the mechanisms involved in IL-7-induced viability and proliferation in T-ALL, identifying CK2 as a master regulator not only of cell-intrinsic but also of growth factor-dependent activation of pro-survival pathways in this malignancy.

0029

Do high systemic cholesterol levels influence the endothelia of target organs to facilitate metastasis?

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The majority of metastatic dissemination occurs through the haematogenous route and factors that alter endothelial properties at distant organs can promote extravasation of cancer cells. It is widely known that high levels of systemic cholesterol lead to atherosclerosis and dysfunctional arterial endothelia. High cholesterol has also been associated with poor prognosis in several

cancers. We have previously shown, in a murine xenograft model of acute lymphoblastic leukemia, that mice fed on a high-cholesterol diet, show increased disease burden and invasion of distant organs, such as the central nervous system and the lungs, compared with mice fed on normal diet. Here we hypothesize that systemic cholesterol modulates the endothelia of target organs of metastasis, to facilitate extravasation. In vitro, we observed that LDL-cholesterol, acting through the LDL-receptor, increases the permeability of endothelial cell monolayers. Most interestingly, in vivo, we show that upon hypercholesterolemia, endothelial cells of the liver, but not the lungs, are enriched in cholesterol. We are now developing a model of liver metastasis through the injection of colorectal cancer cells in the spleen. This will allow us to address the effect of cholesterol-enriched endothelia in the colonization of the liver by cancer cells. Overall, this work will contribute to understand how systemic (endothelial-specific) effects of high cholesterol may promote metastasis formation.

0030

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

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T-cell acute lymphoblastic leukemia (T-ALL) constitutes an aggressive subset of ALL, the most frequent childhood malignancy. Despite successful chemotherapeutic regimens, there are still significant long-term side effects and relapsed patients have dismal prognosis. New therapies are therefore required relying on a better understanding of T-ALL biology. Interleukin-7 (IL-7) is produced by bone marrow and thymus stromal cells. Being essential for normal T-cell development, IL-7 may also promote leukemia expansion. Previously, we showed that IL-7 accelerates T-ALL expansion in vivo (Silva et al, Cancer Res. 2011) and leukemia cell survival and proliferation in vitro by activating PI3K/Akt/mTOR signaling pathway (Barata et al, J Exp Med. 2004), modulating p27kip1 and Bcl-2. However, T-cell lymphomas arising in IL-7 transgenic mice depend on STAT5 activity. Thus, we investigated whether STAT5 could be involved in the IL-7 pro-leukemia effects in human T-ALL cells. Using an IL-7-dependent leukemia T-cell line (TAIL7), we show that IL-7 induces JAK-STAT5 pathway transcriptional activity in a dose- and time-dependent manner. To establish the role of STAT5, we evaluated HPB-ALL cells stably expressing STAT5a shRNA and found that STAT5 is indispensable for IL-7-mediated T-ALL cell growth and viability. To test the potential clinical applicability of these observations, we treated TAIL7 and primary T-ALL cells with pharmacological inhibitors of JAK3 (WHI-P131), STATs in general (parthenolide) and STAT5 (N-((4-Oxo-4H-chromen-3-yl)methylene)nicotinohydrazide). All inhibitors reverse IL-7-induced downmodulation of p27kip1, and cyclin and transferrin receptor (CD71) upregulation. Accordingly, they abrogate IL-7-mediated T-ALL cell viability, growth and proliferation. Notably, Bcl-2 expression was not significantly affected by STAT5 inhibition, suggesting that STAT5 regulates leukemia T-cell survival by an alternative, Bcl-2-independent mechanism. Overall, these results indicate that STAT5 plays a major role in mediating IL-7/IL-7R effects in T-ALL cells, constituting a promising target for therapeutic intervention.

0031

How Widespread are Centrosome Abnormalities in Cancer?

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The centrosome is the major microtubule organizing center in animal cells. Abnormalities in its number and structure have been observed in diverse types of cancer and correlate with genomic instability. Approximately a century ago Theodor Boveri suggested that centrosome abnormalities lead to cancer. However, to this date, a causative link between centrosome abnormalities, which can be classified as numerical (e.g. extra centrosomes) or structural (e.g. longer centrioles), and cancer remains elusive. To determine whether centriole structure changes are a hallmark of cancer, we screened the NCI-60 cell lines for centrosome abnormalities. This is a set of 59 human cancer lines derived from 10 different tissues (brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate and skin) for which gene expression analysis data are publicly available. We developed an algorithm to automatically identify mitotic cells and score centriole number and length in 3D at 'sub-pixel' resolution. This method allows the identification centrioles that are closer to the limit of resolution of the optical light (200 nm). Interestingly, the majority of the cell lines shows both changes in centriole number and size. These results will be useful to unravel the genetic features of centriole abnormalities to better understand their consequences on tumorigenesis.

0032

Anti-tumoral effect of the non-nucleoside DNMT inhibitor RG108 in human prostate cancer cells

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Background: Current therapeutic strategies for advanced prostate cancer (PCa) are largely ineffective. Since aberrant DNA methylation, associated with inappropriate gene silencing, is a common feature of PCa, DNA methylation inhibitors might constitute an alternative therapy. In this study we aimed to evaluate the anti-cancer properties of RG108, a novel non-nucleoside inhibitor of DNA methyltransferases (DNMT), in PCa cell lines.

Methods: The anti-tumoral impact of RG108 in LNCaP, 22Rv1, DU145 and PC-3 cell lines was assessed through standard cell viability, apoptosis and cell cycle assays. Also, DNMT activity, *DNMT1* expression and global levels of DNA methylation were evaluated in the same cell lines. The effectiveness of DNA demethylation was further assessed through the determination of promoter methylation and transcript levels of *GSTP1*, *APC* and *RAR-β2*, by quantitative methylation-specific PCR and RT-qPCR, respectively.

Results: RG108 led to a significant growth inhibition and apoptosis induction in a dose and time dependent manner, for LNCaP, 22Rv1 and DU145. LNCaP and 22Rv1 also displayed decreased DNMT activity, *DNMT1* expression and global DNA methylation. Interestingly, chronic treatment with RG108 significantly decreased *GSTP1*, *APC* and *RAR-β2* promoter hypermethylation levels, although mRNA re-expression was only valid for *GSTP1* and *APC*.

Conclusions: RG108 proved to have a tumor growth suppressor potential in most PCa cell lines tested. This is most likely explained by reversion of aberrant DNA methylation in promoter regions of cancer related-genes silenced in PCa. Nevertheless, additional mechanisms might count for anti-tumoral effects of RG108. *In vivo* studies are now required in order to corroborate these promising results and evaluate the real potential of this compound for PCa therapy.

0033

Assessment of enoxacin effect on cancer growth and microRNA expression in prostate cell lines

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Background: Prostate cancer (PCa) is one of the most incident malignancies worldwide. Although efficient therapy is available for early-stage PCa, treatment of advanced disease is mainly ineffective and remains a clinical challenge. MicroRNA (miRNA) dysregulation is associated with PCa development and progression. In fact, several studies have reported a widespread downregulation of miRNAs in PCa, which highlights the importance of studying compounds capable of restoring the global miRNA expression.

Aim: The main aim of this study was to define the usefulness of enoxacin as an anti-tumoral agent in PCa, due to its ability to induce miRNA biogenesis in a Trans-activator RNA-binding protein (TRBP)-mediated manner.

Methodology: Five PCa cell lines were screened for *TARBP2* mutations by direct sequencing and the protein levels of TRBP were evaluated by Western Blot. The protein levels of TRBP in primary prostate carcinomas were assessed by immunohistochemistry. After exposure of cell lines to enoxacin, cell viability, apoptosis, cell cycle, and cell invasion assays were carried out. A miRCURY LNA™ array was used to determine the impact of enoxacin on the expression of miRNAs. The protein levels of a target of one of the overexpressed miRNAs were assessed by Western Blot.

Results: All PCa cell lines were *TARBP2* wild-type and expressed TRBP protein. Furthermore, primary prostate carcinomas displayed normal levels of TRBP protein. Enoxacin was able to decrease cell viability, induce apoptosis, lead to cell cycle arrest, and inhibit the invasiveness of PCa cell lines. Enoxacin was also effective in restoring the global expression of miRNAs. Moreover, the overexpression of miR-449a, one of the tumor-suppressor miRNAs implicated in PCa, was associated with the downregulation of its target oncoprotein, histone deacetylase 1 (HDAC1).

Conclusions: These results demonstrated that PCa cells are highly responsive to the anti-tumoral effects of enoxacin. Therefore, enoxacin constitutes a promising therapeutic agent for PCa.

0034

Diagnostic and prognostic value of the *IDH1* codon 132 mutation and *MGMT* promoter methylation in Gliomas

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Gliomas are the most common primary brain tumors and their major representative forms are astrocytomas, oligodendrogliomas and ependymomas. Grade IV astrocytomas, usually referred as glioblastoma multiforme, is the most frequent and lethal type. Despite advances in therapeutic approaches, the prognosis of most patients is still extremely poor. Therefore, the identification of molecular markers to improve the clinical outcome of these patients represents an important challenge.

We aimed to assess the frequency and the value of diagnostic and/or prognostic of two markers: (i) somatic mutations in isocitrate dehydrogensase 1 and 2 genes (*IDH1* and *IDH2*) and (ii) the methylation of O6-methylguanine DNA methyltransferase gene *(MGMT),* in a series of Portuguese patients with gliomas.

One hundred and twenty eight patients assisted in the University Hospital of Coimbra were enrolled. Of these, 103 patients with astrocitomas, 15 with oligodendrogliomas, 6 with mixed gliomas and 4 with ependymomas were screened for the presence of somatic mutations in *IDH1* and *IDH2* genes using directed sequencing. Hypermethylation of the promoter region of the *MGMT* gene was evaluated using Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) in 30 glioblastoma patients.

We found seventeen patients with known missense mutations in codon 132 of the *IDH1* gene, including Arg132His (most frequent), Arg132Ser and Arg132Leu. No mutations were found in *IDH2* gene. Our data revealed that *IDH1* mutations are frequent in secondary glioblastomas but rare in primary glioblastomas (100% *vs* 1%, p<0,0001). This mutation seems to be associated with a more favorable prognosis. The *MGMT* promoter was methylated in 67% and unmethylated in 33% of glioblastoma patients. The patients who had the *MGMT* methylated and underwent chemotherapy/radiotherapy showed longer survival (p<0,001).

In conclusion, our data suggested the importance of these markers evaluation, in a routine basis, due to their diagnostic and prognostic value.

0035

Gastric intestinal metaplasia: focusing on CDX2 role and regulation

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Gastric cancer remains a serious health burden worldwide, ranking second as leading causes of cancer-related deaths. Worsening this scenario, gastric cancer survival is globally low and has not significantly improved over the last decades, with treatment being largely palliative. Intestinal metaplasia (IM) of the most relevant pre-neoplastic lesion of the stomach, appearing following *Helicobacter pylori* infection and conferring increased risk for gastric cancer development. However, the molecular networks connecting infection to lesion formation and the cellular origin of this lesion remain largely unknown, and the former has been one of our areas of research. Nevertheless, a more comprehensive understanding of how IM arises and is maintained will be a major breakthrough towards the possibility of developing novel therapeutic interventions improving patient management. After ascertaining the pivotal role played by the intestine-specific homeobox gene *CDX2* in establishing and maintaining IM, where it appears ectopically expressed, it became important to decipher the upstream molecular pathways leading to this aberrant expression. Digging into CDX2 regulation in the context of the pathophysiology of IM has been the main focus of our work over the past few years. We have studied the involvement of the BMP pathway, alone and as a downstream effector of *H. pylori* infection, as well as an autoregulatory loop allowing CDX2 expression perpetuation upon initiation. In addition, we have tackled the issue of the putative reversibility of IM lesions following *H. pylori* eradication. Our work thus provides major contributions to the understanding of IM lesions in the context of the molecular network involved in its establishment and maintenance, with emphasis on CDX2 function and regulation.

0036

Fibronectin overexpression is associated to a more aggressive phenotype - *in vitro and in vivo* colon cancer models

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Reciprocal interactions among normal cells, their mediators, components of the extracellular matrix (ECM) and genetically altered cells regulate all aspects of tumorigenicity. Fibronectin (FN), a multidomain glycoprotein, represents the major component of ECM and is implicated in a variety of cell functions, particularly those involving interactions between cells and ECM. ECM proteolysis is a crucial step during cancer stages and FN susceptibility to proteolytic degradation is well documented. Indeed, several studies have related FN

levels to tumor progression in cancer patients, observing an increase of both FN and FN fragments levels. In parallel, some research has, on the other hand, provided evidence of the protective roles of fragments derived from FN.

Here we exploited the role of FN produced by tumors in modulating tumor development and progression. For this purpose, we generated a stably transfected colon carcinoma cell line (HCT15 cells) which overexpresses FN (and produces elevated amounts of FN) and compared it to its wild type counterpart in well characterized in vitro and in vivo assays.

In vitro, higher proliferation and directional migration rates (in wound healing assays) were observed in FN-transfected cells, as well as a decrease in apoptosis. In vivo, higher FN levels were associated to larger primary tumor masses, more invasion, and, ultimately, a decrease in survival of mice inoculated with FN-transfected cells. Overall, our findings suggest a correlation between higher FN levels and a more aggressive behavior of cells in a neoplastic context (colon cancer). FN seems, therefore, to represent a potential target in therapeutic approaches to cancer.

0037

Role of Cancer Stem Cells in bladder cancer susceptibility to chemo and Natural Killer cells-based therapy.

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Bladder cancer (BC) is characterized by an aggressive phenotype with high propensity for recurrence and/or metastasis, probably related with the presence of Cancer Stem Cells (CSC), hypothesized as the real driving force behind tumor growth, self-renewal and resistance to conventional therapies.

Natural Killer (NK) cells are lymphocytes able to kill a wide range of cancer cells due to its powerful cytolytic activity, being considered suitable candidates for adoptive immunotherapy.

We aim to explore the role of CSC in the susceptibility of BC-cell lines to NK cell mediated-based immunotherapy.

Two human BC cell lines (HT-1376 and UM-UC3) were assayed for their susceptibility to NK cells-induced lysis, using the CD107a-based degranulation assay. The presence of CSC was analyzed using the sphere-forming assay. Cells' chemosensitivity cisplatin (CIS) and methotrexate (MTX) was determined using the MTT-colorimetric assay after 48h incubation with increasing concentrations of drugs.

A subset of CSCs was identified in the HT-1376 cell line in contrast to the UM-UC3 cell line. Surface expression of CD107a in NK cells following co-incubation with BC cell lines increased significantly ($p<0.05$) compared to the baseline activity (17.44±2.17%). Up-regulation of CD107a on NK cells exposed to UM-UC3 cells (59.51±8.17%) was slightly higher as compared with sphere forming HT-1376 cells (43.81±8.65%). MTT results showed that HT-1376 cells are more resistant to CIS and MTX than the UM-UC3 cells. Drugs concentration required to inhibit cell viability in 50% (IC_{50}) for HT-1376 cells was of 7.45±1.20µM for CIS and0.18±0.03µM, significantly higher ($p<0.05$) as compared with the UM-UC3 cell line (CIS:IC_{50}=3.98±0.70µM; MTX:IC_{50}=0.04±0.01µM).

The sphere-forming HT-1376 cells are more chemoresistant than the UM-UC3 cells, probably due to the presence of CSC. Both BC cell lines are susceptible to NK cell-mediated cytotoxicity, independently of the presence of CSC. This might be an alternative approach to eliminate drug-resistant stem cells and prevent tumor recurrence.

0038

***BRAF* and *NRAS* mutations in thyroid carcinomas following childhood scalp irradiation**

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Introduction - Exposure to ionizing radiation at young age is known as the strongest risk factor for thyroid carcinoma development, namely papillary thyroid carcinoma (PTC). The most frequent genetic alterations associated with PTC are *BRAF* mutations, *RAS* mutations and *RET/PTC* rearrangements. The aim of this study was to evaluate those genetic alterations in PTCs following childhood scalp irradiation.**Material and methods** - We were able to recover 60 thyroid tumours from 47 individuals irradiated in childhood for tinea capitis scalp epilation, being 33 malignant and 27 benign. The malignant lesions included 11 conventional PTCs (cPTC), 2 metastized lymph nodes with cPTC growth pattern, 19 follicular variants of PTC (PTC/FV) and one oncocytic

variant of PTC.The lesions were screened for the hotspot BRAF mutation at nucleotide 1799 (residue 600) ($BRAF^{V600E}$ mutation) and for *NRAS* mutations. The study of *RET/PTC* rearrangements is ongoing.**Results** - We detected the presence of the $BRAF^{V600E}$ mutation in 7 out of 13 (54%) cPTC and 2 out of 19 PTC/FV (11%) thus showing that the mutation is significantly more prevalent in cPTC than in PTC/FV (p=0.015). One of the 19 PTC/FV showed the rare $BRAF^{VK600E-1E}$ mutation, described by our group in PTC/FV. *NRAS* mutation was present in one case of PTC/FV (3%). None of the benign lesions - follicular adenomas - presented *BRAF* or *NRAS* mutations.**Conclusion** - Our results show that the prevalence of $BRAF^{V600E}$ mutation in PTCs from patients subjected to childhood scalp irradiation is similar to that observed in series of sporadic PTCs (36-69%), but it is higher than that previously reported in other settings of PTCs from irradiated individuals, both with internal or external radiation (4-24%). As far as we are aware this is the first study of *BRAF* and *NRAS* mutations in thyroid carcinomas arising in patients with tinea capitis scalp irradiation.

0039

Role of Apoptotic Pathways in Chemoresistance of Osteosarcoma Cancer Stem Cells

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Background: Osteosarcoma (OS) is the most common primary malignant bone tumour in children and adolescents, which results from genetic and epigenetic mutations during differentiation of mesenchymal stem cells in osteoblasts. Recent observations showed that OS contains a subset of cancer stem cells (CSC), which are responsible for initiation and progression of tumor as well as for the resistance to conventional therapies. We aimed to explore the role of the intrinsic apoptotic pathway in CSC response to doxorubicin (DOX).

Methods: CSC were isolated from the human OS cell line MNNG/HOS using the sphere formation assay and then incubated with DOX for 48h. The cytotoxicity of DOX was evaluated considering effects on cell viability (MTT assay), proliferation (BrdU Assay) and apoptosis (TUNEL assay). Expression levels of the anti-apoptotic proteins Bcl-2 and Bcl-X_L, pro-apoptotic proteins Bax and Bak, and caspase-3 was analyzed by Western blot.

Results: The isolated CSC were relatively more resistant to DOX compared to MNNG/HOS cells. DOX concentration required to inhibit cell viability by 50% (IC_{50}) for CSC (2.21±0.50μM) was significantly higher ($p<0,05$) than that of parental cells (0.67±0.17μM). The IC_{50} that inhibits cellular proliferation was of 0.03±0.01μM for CSC and 0.19±0.06μM for MNNG/HOS cells ($p<0.05$). MNNG/HOS cells showed a dose-dependent increase in the percentage of apoptotic cells ranging from 1% to 21% in opposite to CSC which percentage remained approximately constant, not exceeding the 3%. DOX induced a significant increase in the expression levels of the anti-apoptotic proteins Bcl-2 and Bcl-X_L and a concomitant decrease in the expression of the pro-apoptotic protein Bak and caspase 3 in CSC.

Conclusions: MNNG/HOS contains cells with stem-like properties that are relatively more resistant to DOX than their parental cells. Overexpression of Bcl-2 and Bcl-X_L concurrently with the reduction of Bak appears to contribute to the higher resistance of CSC to DOX-induced apoptosis.

0040

Overexpression of ST3Gal-IV induces activation of cell signaling pathways and alterations in gastric cancer cell line phenotype

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The overexpression of Sialyl-Lewis antigens is a common feature in cancer. The presence of these glycan structures has been implicated in biological features of cancer cells and are potential cancer biomarkers (1). We have previously demonstrated, in stable transfected gastric cell lines, that ST3Gal-III and ST3Gal-IV are involved in the production of SLe^a and SLe^x antigens in gastric carcinoma cells (2). In the present study we assessed the biological behavior of these cells in classical *in vitro* assays and in the *in vivo* chicken chorioallantoic membrane (CAM) model. The *in vitro* characterization of the cell lines showed that cells expressing ST3Gal-IV are more invasive although no differences in cell proliferation were observed. In addition, the ST3Gal-IV expressing cells showed a significant increase in cell invasion in the *in vivo* CAM model, while no differences were observed in angiogenesis and tumor growth. Constitutive activation of receptor tyrosine kinases was observed.

We further characterized the cell proteome and secretome in ST3Gal-III and ST3Gal-IV transfected cells. We have observed differences in protein expression and proteins carriers of SLe^a and SLe^x were identified. The cell secretome showed, by western blot, that the transfected cells are secreting proteins with SLe^a and SLe^x antigens and the proteins identified are being further evaluated as possible new biomarkers.

Our results demonstrate that these sialylated glycans contribute for the invasive phenotype of gastric cancer cells, playing a role in the process of cancer cell invasion.

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0041

Development of clonal cancer cell escape variants as the basis for relapse in acute myeloid leukaemia

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Post-therapeutic relapse limits most acute myeloid leukemia (AML) treatments. Studies comparing matched primary and chemotherapy-resistant AML genomes indicate that clinical tumor escape variants arise due to clonal evolution. Preclinical systems that feasibly evaluate clonal architectures during cancer treatments/recurrence are nevertheless lacking. Therefore, we develop novel *in vivo* systems to analyse human AML clonal evolution in response to individually optimized therapies. We established a zebrafish xenotransplantation model by injecting 100-200 CM-Dil labelled HEL cells (human erythroleukemia cell line) in larvae (maintained at 34^{o}C) and monitored tumor burden over a week by intravital florescence microscopy. Our mouse xenotransplantation model is based on intravenous injections of 10^4-10^7 HEL cells, transduced to express luciferase and GFP, into irradiated Rag2^{-}/γc^{-}/$^{-}$ mice. Tumor establishment and progression was evaluated longitudinally by bioluminescence and flow cytometry analysis (% GFP^{+} cells in peripheral blood (PB)). The disease development constantly showed initial AML expansion in the bone marrow, followed by progressive spread to the PB, spleen, liver and lungs; thus recapitulating clinical AML pathophysiology. We will now introduce barcoded primary patient-derived AML into these xenotransplantation models and employ them to study *in vivo* clonal evolution upon post-therapeutic AML relapse. A coordinated system will enable this: 1) High-throughput drug screening in zebrafish, to define an optimal personalized treatment for each individual primary AML; 2) Cellular barcoding technology, which labels thousands of AML cells in each sample with individual traceable molecular tags (noncoding DNA) that allow tracking of progeny development over time; 3) *In vivo* modelling of primary AML development in response to optimal therapies, using the mouse xenotransplantation model. Considering the clinical importance of clonal evolution during cancer treatments, this project will provide a valuable experimental system to interactively study the effects of different therapeutic regimens on AML clonal architectures and evolutionary events underlying emergence of therapy-resistant escape variants mediating relapse.

0042

Phthalocyanine galacto-dendritic conjugate: decreases the galectin-1 protein levels and induces phototoxicity in bladder cancer cells

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Introduction: Photodynamic therapy (PDT) has been applied to improve the treatment of several cancers, especially the ones occurring in accessible cavities, like the bladder. PDT combines a photosensitizer (PS), light and molecular oxygen to generate reactive oxygen species (ROS). Our research group recently reported the synthesis of a powerful PS: a phthalocyanine-PcGal$_{16}$-decorated with galactose units, which can be recognized by galectins overexpressed in cancer cells. PcGal$_{16}$ produces ROS, is photo-stable and may be effective in treating deep-seated tumors. The aim of this work was to evaluate the putative beneficial effects of this PS against bladder cancer.

Methods: UM-UC-3 and HT-1376, human bladder cancer cell lines, were used to perform in vitro studies. The uptake of PcGal$_{16}$ by cancer cells was evaluated by fluorescence spectroscopy and microscopy. The distribution of galectin-1 before/after PcGal$_{16}$ uptake was determined by immunofluorescence. The cytotoxicity before/after PcGal$_{16}$-PDT was determined by Trypan blue exclusion and MTT colorimetric assays. PDT-induced ROS was detected using the dichlorofluorescein probe.

Results: The uptake of PcGal$_{16}$ by cancer cells increased in a time/concentration dependent manner. Fluorescence microscopy revealed that PcGal$_{16}$ is distributed throughout the cytoplasm of cells. Incubation of cancer cells with PcGal$_{16}$ in darkness did not induced toxicity but induced a decrease in galectin-1 which is co-localized with PcGal$_{16}$. Photo-toxicity induced by PcGal$_{16}$-PDT was exhibited in a dose/time dependent manner and it was associated with ROS generation.

Conclusion: Galectin-1 is a promising molecular target for cancer therapy due to its contribution to tumor progression and resistance after conventional cancer therapy. The PcGal$_{16}$ "dark" mode of action by decreasing galectin-1 after its uptake in cancer cells, as well as its

photo-toxicity prompted us to envisage $PcGal_{16}$ as a novel anticancer drug candidate.
This work was supported by FCT (PEst-C/SAU/UI3282/2011) and the project PTDC/CTM/101538/2008, and ACIMAGO (Ref. 12/12).

0043

***Helicobacter pylori* and the BMP pathway regulate CDX2 and SOX2 expression in gastric cells**

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Introduction: Helicobacter pylori infection is the main risk factor for intestinal metaplasia (IM) and gastric cancer development. IM is a pre-neoplastic lesion, induced by the transcription factor CDX2, where the gastric mucosa is converted to an intestinal phenotype. We previously demonstrated that key elements of the BMP pathway co-localize with CDX2 in IM and upregulate CDX2 expression in gastric cell lines. These observations, together with the hypothesis that CDX2 could be repressed by SOX2, led us to test whether H. pylori, through BMPs, SOX2 and CDX2 could participate in a molecular network critical for the development of IM.

Materials and Methods: AGS cells with and without SMAD4 knock-down were co-cultured with H. pylori or BMP2 to assess the expression of BMP pathway members as well as CDX2 and SOX2 by qPCR and Western blot. Proximity ligation assay was also performed to evaluate SMAD proteins interaction. Immunohistochemistry and Western blot were performed in gastric samples from mice infected with Helicobacter spp. to measure Smad4, pSmad1/5/8, Cdx2 and Sox2 expression in vivo.

Results: Increased expression and activity of the BMP pathway accompanied by CDX2 upregulation and SOX2 downregulation was observed in AGS cells co-cultured with H. pylori or BMP2. These effects were impaired by downregulation of the BMP pathway. Finally, infected mice present BMP pathway upregulation, focal Cdx2 expression and decreased Sox2.

Conclusion: These results provide a novel link between H. pylori infection and the BMP pathway in the regulation of intestinal and gastric-specific genes that might be relevant for gastric IM.

0044

Cholesterol Promotes Breast Cancer Growth

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Obesity is as a breast cancer (BC) risk factor. Despite major modifications of lipid metabolism during obesity, little is known about the role of plasma cholesterol in BC. In this study, we ask if a cholesterol enriched macroenvironment promotes BC progression.

1) Lipid profile (Total cholesterol, Low Density Lipoprotein(LDL), High Density Lipoprotein, triglicerides) of 244 women, with BC, without previous treatment or familiar history and not taking lipid-lowering or anti-diabetic drugs, were determine. Statistical analysis was performed using SPSS version 19.0.

2)Proliferation, migration adhesion assays of BC cell lines (MDAMB231, HTB20 and HTB126) exposed to LDL (100ug/ml, 24h). Microarray analyses (Affymetrix GeneChips) were done in control and LDL conditions, and IPA Ingenuity Systems was used to explore networks and relevant biological interactions.

3)Orthotopic mice model (MDA-MB 231cells/ BAlb-SCID) were subject to a 40-day high cholesterol diet (HD). Standard diet fed-mice (ND) were used as control. Primary tumor and metastasis target organs were collected for histopathology.

Systemic levels of LDL correlates positively with tumor size (rho=0,199, *p*0,002), T and clinical stages.

Tumor size increases significantly across LDL level terciles. Patients in the LDLT3 are more likely to have positive lymphovascular invasion, lymph node metastization and be diagnosed in advanced stages. There are no differences in lipid profile, between subtypes, but tumors of patients in the LDLT3 are more commonly HER2-neu positive.

Multivariate logistic regression found that the LDL>117mg/dl is a predictor factor to tumor size≥20mm, even when adjusted to BMI and age.

In vitro, LDL, induces cell proliferation (2,6 fold), migration (control 25%; LDL:100%, p0,007), loss of adhesion and over expression of AKT, MAPK and Her2-neu signaling pathways.

HD showed larger tumors with higher proliferation rate (Ki67:ND 25,16%; HD 51,7%, *p*0,02).

In conclusion, tumors in LDL enriched macroenvironment are in survival advantage. Initial molecular studies suggest induction of proliferative pathways and may support the importance of LDL control to tumor treatment.

0045

miR-34a AND miR-125b EXPRESSION IN HPV INFECTION AND CERVICAL CANCER DEVELOPMENT

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Background: Clinicians are still demanding for predictive/prognostic biomarkers for HPV infection and cervical lesions progression. Recent studies suggested microRNAs as possible biomarkers of HPV-associated cancers, and therefore we aimed to characterize miR-125b and miR-34a expression in cervical samples.

Methods: miR-125b and miR-34a expression was determined by qRT-PCR methodology in 110 women with different cervical lesions: normal epithelium with (n=20) and without (n=29) HPV infection: LSIL (n=28); HSIL (n=21) and CIS/ICC (n=12).

Results: We observed a two-fold increased miR-125b expression among normal cases with HPV infection ($2^{-\Delta\Delta Ct}$=2.11; *p*=0.038). Data also showed a trend to down-regulation of miR-25b in LSIL and HSIL cases and a significant decreased expression in women with either CIS or ICC ($2^{-\Delta\Delta Ct}$=0.75; $2^{-\Delta\Delta Ct}$=0.78; $2^{-\Delta\Delta Ct}$=0.33 and $2^{-\Delta\Delta Ct}$=0.23, respectively). miR-34a expression analysis revealed an increased expression among women with normal cervix and HPV infection ($2^{-\Delta\Delta Ct}$=1.69; *p*=0.049) but no significant change was observed for LSIL, HSIL or CIS/ICC.

Conclusion: This is the first study to characterize the expression of miR-125b and miR34a in cervical samples. Results showed that while miR-34a expression remains constant, miR-125b expression is significantly changed in the different cervical lesions and its levels should be further investigated as possible predictive/prognostic biomarkers using a non-invasive approach.

0046

IS HPV-16 INTEGRATION A PREDICTOR MARKER OF CERVICAL LESIONS?

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Objective: The persistent infection of *human papillomavirus* (HPV) has been established as the main etiological factor for the development of squamous intraepithelial lesions of the cervix which may progress to invasive carcinoma. The integration of HPV genome into the host´s genome is considered the hallmark of HPV-associated carcinogenesis. However, the significance of HPV physical status detection remains unclear. The aim of this study was to characterize the physical status of HPV-16 in samples with different histological classifications.

Methods: We have selected 53 cervical specimens from women with different histological classification (7 normal, 15 atypical cells of undetermined significance (ASC-US), 12 low-grade squamous intraepithelial lesion (LSIL), 15 high-grade squamous intraepithelial lesions (HSIL) and 4 invasive cervical carcinoma (ICC)) that have been identified with HPV-16 infection. The physical status of HPV16 was analyzed using a multiplex Real-time PCR methodology. HPV-16 status classification was based on the principle that, when integration occurs, the E2 gene is partially or totally disrupted while the E6 gene remains intact.

Results: In this study, the prevalence of HPV16 integration was of 26.4% (14/53, 13 mixed forms and 1 integrated only). Prevalence of HPV-16 integration among different cervical lesions was 28.6% (2/7) in samples without cytological lesion, 13.3% (2/15) in ASCUS, 33.3% (4/12) in LSIL, 33.3% (5/15) in HSIL and 25.0% (1/4) in ICC. Additionally, we no found statistical significant differences in HPV-16 integration distribution among the histological specimens (*p*=0.735).

Conclusion: Our study revealed that HPV 16 integration is not exclusive event of HSIL/ICC. It was not possible to detect integrated forms in all cases of HSIL/ICC. This fact reveals the need to reconsider the role of viral genome integration in HPV-associated carcinogenesis and suggests the requirement of further studies, preferably cohort studies, to follow-up normal, ASC-US and LSIL cases which present HPV integration and evaluate their progression.

0047

Role of sodium lactate (NaLac), Epidermal Growth Factor (EGF) and c-Myc in the expression of Monocarboxylated Transporter 1 (MCT1) in uterine cervix cancer

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Metabolism is a hallmark for cancer. Frequently, tumors have different metabolism from normal tissues. Although NaLac is considered a metabolic-waste-product, several studies show that it fuels some tumor cells metabolism. In cervix, NaLac rich microenvironment plays a role in cancer cells selection due to their ability of consuming NaLac. In cancer, MCT1 is expressed and mediates NaLac uptake and release. Our studies showed that SiHa proliferates and migrates more when cultured with NaLac. By NMR we observed that SiHa metabolises NaLac. EGF and c-Myc are relevant in cervical cancerigenesis.

Our objective was to evaluate the role of NaLac and EGF through c-Myc in MCT1 expression in cervical cancer: adenocarcinoma(AC;HeLa) and squamous-cell-carcinoma(SCC;SiHa). SCC and AC were evaluated for MCT1 expression and c-Myc and chromosome 8 (chr8) copies.

MCT1 was detected by immunofluorescense (cell lines) and immunohistochemistry (tissues). In HeLa, EGF but not NaLac increases MCT1 expression. In SiHa, EGF and NaLac upregulate MCT1 expression. By luciferase-reporter-gene-assay, EGF and NaLac increase MCT1 promoter activity. By chromatin-immunoprecipitation(ChIP), c-Myc binds respectively more and less MCT1 promoter in HeLa and SiHa grown with NaLac and EGF. By fluorescent-*in-situ*-hybridization-(FISH), HeLa has c-Myc amplification and SiHa chr8-tetrasomy. In AC, 2(16,7%) were MCT1(+) and chr8-disomic and 10(83,3%) were MCT1(-),8-chr8-disomic and 2-chr8-aneusomic. In 47 SSC, 32(68%) were MCT1(+),15-chr8-disomic, 4-chr8-trisomic,7-chr8-aneusomic and 6-chr8-aneusomic-c-Myc amplified and 15(32%) were MCT1(-),9-chr8-disomic,3-chr8-trisomic and 7-chr8-aneusomic. In normal cervix, 90% of cases express MCT1 in basal-squamous-cells while glandular-epithelium is MCT1(-).

NaLac, EGF and c-Myc regulate MCT1 expression in HeLa and SiHa. C-Myc acts as activator and as repressor. In SSC 74% of cases with cytogenetic alterations are MCT1(+). In SCC, MCT1 can be a suitable therapeutic target, since it is expressed in the majority of cases and as we observed *in vitro* there is a trend between the expression of MCT1 and the proliferation rate of cells.

0048

Mitotic Misregulation and Aging

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Cancer is primarily an age-related disease. How aging promotes late-life cancer remains largely unknown. Our working hypothesis is that cellular aging compromises mitotic fidelity leading to chromosomal instability. To address this issue we have ascertained the effect of both natural aging and replicative senescence (in vitro model of aging) in mitotic progression using long-term time-lapse phase contrast microscopy. We performed comparative analysis between dermal fibroblasts derived from neonatal, young-age and old-age humans and mice, as well as between low passage and high passage/senescent fibroblasts. We found a significant mitotic delay in both aged and replicative senescent adult fibroblasts. High resolution time-lapse microscopy of those fibroblasts indicated a prometaphase delay, which was shown to be dependent on the activation of the spindle assembly checkpoint (SAC). Consistently, fixed cell analysis revealed increased number of prometaphase cells with spindle defects and chromosome misalignment. Furthermore, we found a higher frequency of aneuploid and polyploid cell karyotypes. Our working model is that by inducing chromosomal instability, aging might lead to potential loss of tumor suppressor pathways.

0049

Frequency and survival of second primary cancers in North Portugal - a population-based assessment

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Background: A dramatic increase in cancer survivorship and in the frequency of second primary cancers (SPC) has been observed in the latest decades, urging the investigation of their burden at a population level. We aimed to quantify the proportion of SPC among the incident cases in the North Portugal and to describe their survival.
Patients and methods: We identified all SPC (excluding skin non-melanoma) registered by the North Region Cancer Registry (RORENO) in 2000-2003, according to the

International Association of Cancer Registries and the International Agency for Research on Cancer guidelines. We classified tumors diagnosed more than 2-months after a first primary cancer (FPC) as metachronous. The observed survival was computed using vital status at December 2010.
Results: A total of 1,607 SPC (3.8% of all cancers) were registered (77.9% metachronous). The most frequent metachronous SPC topographies and corresponding most frequent FPC were colon (12.2%; FPC: prostate, breast and stomach), lung (10.5%; FPC: bladder, stomach and colon) and stomach (9.7%; FPC: prostate, breast and bladder). The overall 5-year survival of metachronous SPC was 47.4%; within the subgroups with higher (63.1%) and lower survival (31.1%) there were no significant differences accross groups of FPC with expectably different survival.
Conclusions: The proportion of SPC was the anticipated for a registry with approximately one decade of activity. The most common cancers in the general population were also frequent metachronous SPC, while the most frequent FPC were high incidence and survival cancers. The survival of metachronous SPC did not vary with the survival expected for the FPC.

0050

Effects of SMYD3 in prostate carcinogenesis

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Prostate cancer (PCa) is a leading cause of cancer-related morbidity and mortality worldwide. Because the currently used parameters to predict the aggressiveness of a given PCa are rather imperfect, it is extremely important to identify new prognostic markers to define the most appropriate therapeutic strategy. Alteration of chromatin modification patterns have been attributed to altered expression or activity of key chromatin-modifying enzymes, including histone methyltransferases (HMTs). Deregulation of some HMTs, namely *EZH2* and *CARM1,* has been already associated with prostate carcinogenesis, however the importance of other members of HMTs is poorly understood. Therefore, our main goal was to clarify the role of HMTs altered expression in PCa development and progression and to translate those findings into clinically useful tools for PCa patients management.To achieve this goal, gene expression of 37 HMTs was investigated by *TaqMan® Arrays Plates* analysis in 10 primary PCa and 5 Morphological Normal Prostate Tissue (MNPT). This data was further confirmed in a larger and independent series of 150 primary PCa and 15 MNPT, and their mRNA expression levels were correlated with clinicopathological data. We found that *SMYD3*, an H3K4 methyltransferase, was significantly overexpressed in tumors especially in advanced stages (pT3b). Moreover, we stably silenced *SMYD3* in a PCa cell line, LNCaP, and assessed the effects in cell viability, apoptosis and migration through standard assays. SMYD3 knockdown did not affect global H3K4 methylation levels but appears to impact in selective promoter regions. Although the absence of SMYD3 did not alter cell viability, it led to a significant decrease of PCa cells migration rate, as well as to an increase in apoptosis.Even though additional studies in other PCa cell lines are required, our findings suggest that SMYD3 plays a important role in prostate carcinogenesis and therefore it may be useful as a therapeutic target in aggressive PCa.

0051

Mitotic Misregulation and Aging

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Cancer is primarily an age-related disease. How aging promotes late-life cancer remains largely unknown. Our working hypothesis is that cellular aging compromises mitotic fidelity leading to chromosomal instability. To address this issue we have ascertained the effect of both natural aging and replicative senescence (in vitro model of aging) in mitotic progression using long-term time-lapse phase contrast microscopy. We performed comparative analysis between dermal fibroblasts derived from neonatal, young-age and old-age humans and mice, as well as between low passage and high passage/senescent fibroblasts. We found a significant mitotic delay in both aged and replicative senescent adult fibroblasts. High resolution time-lapse microscopy of those fibroblasts indicated a prometaphase delay, which was shown to be dependent on the activation of the spindle assembly checkpoint (SAC). Consistently, fixed cell analysis revealed increased number of prometaphase cells with spindle defects and chromosome misalignment. Furthermore, we found a higher frequency of aneuploid and polyploid cell karyotypes. Our working model is that by inducing chromosomal instability, aging might lead to potential loss of tumor suppressor pathways.

0052

In Vitro and in Vivo Targeting of Chronic Lymphocytic Leukemia Using CX-4945, a Clinical-Stage CK2-Specific Inhibitor

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Despite important therapeutic advances, chronic lymphocytic leukemia (CLL) still requires more efficient and specific treatment regimens. We previously demonstrated that CLL cells display overexpression/hyperactivation of the protein kinase CK2, which is essential for their viability. Here, we tested the legitimacy of CK2 as a CLL therapeutic target using CX-4945, a potent and highly specific orally-available ATP-competitive inhibitor of CK2 that is undergoing phase I/II clinical trials for solid tumors and multiple myeloma. We show that CX-4945 decreases the viability and proliferation of CLL cell lines and promotes cell death in all tested primary CLL samples. This effect is time- and dose-dependent, and not overruled by the presence of stromal cells. Moreover, sensitivity to CX-4945 correlates with higher percentage of malignant cells in the blood, Binet stage B/C, higher plasma β2 microglobulin levels, higher proliferation rate (LDT < 12 months), and need for treatment. We previously showed that CK2 phosphorylates and thereby inactivates the tumor suppressor PTEN in CLL cells, leading to the hyperactivation of PI3K signaling pathway. Accordingly, incubation of CLL cells with CX-4945 results in PTEN activation and a concomitant decrease in the activity of PI3K downstream targets Akt and PKC. Furthermore, CX-4945 significantly delays tumor growth in a mouse model where MO1043 CLL cells were inoculated subcutaneously into Swiss Nude mice. Notably, CX-4945 is as effective as fludarabine when used as a single agent, and the combination of the two drugs is significantly more effective than fludarabine alone. No significant toxicities were observed. Overall, our data indicate that pharmacological inhibition of CK2 is a promising therapeutic strategy in CLL that may be of special benefit to patients with aggressive and advanced stage disease. These studies pave the way to the development of clinical trials using CX-4945 or other CK2 antagonists to manage CLL.

0053

Breast Cancer on São Miguel Island, Azores 1982-2010: trends in incidence, survival and mortality

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Introduction: Breast cancer is by far the most frequent cancer among Azorean women. To reduce the limitation of having, from an epidemiological perspective, a small population at risk we looked for trends in incidence, survival and mortality on the most populous of the Azorean islands along almost 30 years. **Methods:** All breast tumours initially diagnosed between the 1st January 1983 and the 31st December 2010 on São Miguel island were included in the statistical analysis. Crude and standardised (direct method, European population) incidence and mortality rates were computed. Net survival at 3- and 6-months, and 1-, 3- and 5-years of follow-up was estimated according to the method recently developed by Maja Pohar and collaborators. Excess mortality was estimated by using a Poisson assumption for the observed number of deaths. Software STATA version 10 was used in the statistical analysis. Trends were analysed trough 'Joinpoint' Program from the Statistical Research and Applications Branch of the US NCI. **Results:** A total of 1130 new cases of breast cancer were diagnosed in 1983-2010 and 410 women died from it in the same period. The ASR has increased from 51.8 to 82.0/100,000, with an APC of 2.1% (95% CI 1.2-3.0) a year. No significant trends were seen on breast cancer mortality on São Miguel. Age-standardised net survival at 5-year of follow-up improved gradually from 89.2% (50.2-98.1) for women diagnosed during 1983-1996 to 93.0% (77.6-98.0) for women diagnosed during 2004-2010. Women diagnosed with breast cancer during 2004-2010 experienced only 57% (0.35-0.92) of the excess mortality experienced by those diagnosed during 1983-1989. **Conclusions:** An increase in diagnostic activity together with changes in demography, lifestyles and reproductive factors could have contributed to an increase in incidence. The rate (hazard) by which women with breast cancer die due to their cancer has slowed down in more recent years.

0054

Downregulation of CDX2 expression in gastric cells using chitosan/siRNA nanoparticles - A strategy to revert gastric intestinal metaplasia

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INTRODUCTION: Gastric intestinal metaplasia (IM) is a pre-malignant condition associated with increased risk of gastric adenocarcinoma. *Helicobacter pylori* eradication is

not able to revert or stop progression of IM in all patients. CDX2 is the key molecular mediator of intestinal differentiation, both in intestine and ectopic foci. CDX2 regulates its own expression and is bound to its own promoter in the mouse intestine and in human IM, being hypothesised that this mechanism is crucial for the maintenance of the intestinal phenotype, making CDX2 an appealing therapeutic target.

AIMS AND METHODS: To design and optimize a nanoparticle (NP)-based delivery system of siRNA directed to CDX2 in gastric cell lines (AGS and IPA220), using chitosan (CH) - a natural, biodegradable cationic polymer with mucoadhesive properties - modified with imidazol (CHimi). NPs with different CHimi to siRNA ratios (N/P, the molar ratio of primary amines to phosphate groups) were prepared. The NP formulation was optimized in terms of siRNA complexation capacity (siRNA retardation assay), NP size (dynamic light scattering) and charge (electrophoretic mobility). NPs cytotoxity was evaluated using a resazurin-based assay. CDX2 downregulation was assessed at mRNA and protein levels. Lipofectamine^{TM}/siRNA complexes were used as controls.

RESULTS: CHimi with a substitution degree of the primary amines of 10 and 16% was obtained. An N/P ratio of 50 rendered the preparation of NPs with a positive net charge (+33V) and an average size<500 nm, able to fully complex the total amount of siRNAs. A 50% decrease in the CDX2 protein was obtained with NPs, without compromising cell viability.

CONCLUSION: We report for the first time a NP-based delivery system directed to CDX2, that can be envisioned as a therapeutic strategy to revert gastric IM *in vivo*.

0055

NMR METABOLOMICS OF LUNG CANCER TISSUES UNVEILS METABOLIC SIGNATURES OF MALIGNANCY AND HISTOLOGICAL TYPE

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Altered metabolism of tumour cells is currently recognized as an emerging cancer hallmark, being at the basis of the metabolomics strategy to find new biomarkers of disease onset and progression, as well as new therapeutic targets.

In this work, the metabolic composition of lung tumour tissues has been investigated by ^{1}H and ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy, to unveil, respectively, alterations in metabolites and phospholipids, compared to non-involved adjacent (control) tissues. Multivariate analysis of ^{1}H NMR spectra, obtained by High Resolution Magic Angle Spinning (HRMAS) analysis of 46 sample pairs, enabled tumour and control tissues to be clearly differentiated (sensitivity and specificity >95%). This distinction was based on variations in metabolite levels reflecting enhanced glycolysis and perturbed lipid metabolism in tumours, together with changes in osmotic regulation, nucleotide metabolism and antioxidant activity. ^{31}P NMR analysis of selected tissue pairs further revealed significant changes in the phospholipid profile: lower levels or absence of phosphatidylethanolamine and phosphatidylserine, together with two new signals suggesting structural changes in the tumours' phospholipids. Furthermore, tumour histological sub-types showed differences in both metabolites and phospholipids, providing new information on the biochemical alterations accompanying morphological changes. Overall, in tandem with the results obtained by metabolic profiling of biofluids (blood plasma and urine) from the same patients and a control group of healthy volunteers, these results highlight the potential of NMR metabolomics in lung cancer diagnosis and follow up.

0056

Metabolic phenotypes associated with distinct androgen-responsive conditions can be a valuable tool for diagnostic and therapeutic options in prostate cancer.

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Prostate cancer is characterized by a transition from an androgen-dependent to androgen-independent phenotype and progresses from precursor lesions (PIN lesions) to localized carcinoma and finally can lead to a metastatic disease, which is often lethal. How metabolic alterations vary according to this different stages of the disease is poorly understood.

In this work we aimed to study a range of key metabolic proteins from non-neoplastic tissue to PIN lesions, localized tumour and finally metastasis in order to understand the most importance of metabolic alterations during prostate cancer initiation and progression, assess the glycolytic behavior of different models of prostate cell lines and finally correlate all with clinical and pathological information in order to observe if the study of metabolic phenotypes could represent a valuable tool for diagnostic and therapeutic possibilities.

For this purpose we studied the expression of 15 key metabolic proteins involved in glycolytic and lipidic β-oxidation pathway and correlate the findings with clinical and pathological data of 480 patients. Additionally, we used different cell line models to evaluate the levels of glycolytic metabolism. Finally we studied the viability of the different cell lines when treated with α-cyano-4-hydroxycinnamate, a classical inhibitor of monocarboxylate transporters, Thioridazine a lipidic β-oxidation inhibitor and Phytanic Acid a long branched chain fatty acid.

The observed results indicated that important differences in the metabolic pathways from non-neoplastic to metastatic disease are visible and have predictive value and also showed that important differences exist between cell line models and this differences are more evident between dependent and independent androgen models. This points to the idea that enlightenment of the metabolic mechanisms during prostate cancer initiation and progression could represent a valuable tool for the design of new diagnosis and therapeutic options.

0057

How strong is the association between environmental exposures and gastric cancer?

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Background: Environmental risk factors for gastric cancer (GC) are potential targets for cancer prevention and control strategies. The quantification of the burden of GC, as well as the impact of such measures, depends on the magnitude of the associations and the frequency of the exposures in different settings.

Aim: To review the literature on summary relative risk (RR) estimates for the association between GC and its main environmental determinants.

Methods: We conducted a systematic review of meta-analyses addressing the association between *Helicobacter pylori* infection, smoking, salt, red meat, fruits and vegetables intake, and GC or precancerous lesions.

Results: *H. pylori* infection increases the risk of chronic atrophic gastritis (CAG) by 5-fold and more than doubles the risk of GC (RR range: 1.92-3.80), which is further increased in those infected with CagA-positive strains (RR range: 1.26-3.63). *H. pylori* eradication reduces the risk of GC (RR=0.65, 95% confidence interval [CI]:0.43-0.98), with significant reduction in CAG but not for intestinal metaplasia (IM). Current smoking increases the GC risk by nearly 50% (RR range: 1.53-1.69), and is also increased among ex-smokers (RR=1.34, 95%CI:1.22-1.47). Salt intake is associated with a higher risk of IM (RR=1.53, 95%CI:0.72-3.24), and both salt and red meat consumption increase the risk of GC (RR=1.68, 95%CI:1.17-2.41 and RR=1.07, 95%CI:1.03-1.11, respectively). Overall, fruits and vegetables intake decrease the risk of GC (RR range: 0.53-0.89 and 0.62-0.98, respectively).

Conclusions: This study presents a summary of the most robust evidence on the role of environmental exposures in gastric carcinogenesis. The protective effect of fruits and vegetables intake is weaker than initially expected. An estimation of the most likely lag times between exposure to these determinants and occurrence of cancer is precluded by the less abundant evidence on the relation with gastric precancerous lesions and on the impact of interrupting the exposure to risk factors.

0058

Biomarkers in oral cancer: The border between the research and the clinical practice

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Introduction: Oral cancer is a great public health problem in the world. These tumors display a great genetic and biologic heterogeneity with alterations in almost all chromosomes, nevertheless some chromosomal regions are appointed as consistently altered. Besides all the research in this field, only a few genes associated with oral cancer have been identified. Therefore, until now it is not clear which genetic imbalances already identified can be used individually or combined, in order to predict the clinical outcome of the oral tumors. In light of the above, the main goal of this study was the characterization of the genomic profile of oral tumors from patients with oral cancer diagnosis through the application of whole genome array-Comparative Genomic Hybridization (aCGH). **Methods:** Biopsies of tumor were acquired from 8 patients. Healthy donors were used as controls. The aCGH was performed using an Agilent oligonucleotide microarray 4x180K. **Results:** With this whole genome approach we detected imbalances in all chromosomes. However, the most commonly losses and gains are detected in specific chromosomes regions. We identified 8815 genes with loss and 1409 genes with gain. Some of these chromosomal regions and genes are already associated

with oral cancer and others play an important role in tumorigenesis development. **Conclusions:** With this approach we identified several genetic alterations consistent with those described in the literature as associated with oral cancer, as well as some genes with strong possibility to be key genes in the oral carcinogenesis. This study also highlights some putative new biomarkers with possible diagnostic and prognostic value.

0059

Molecular subtyping of primary prostate cancer reveals specific and shared target genes of different ETS rearrangements

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INTRODUCTION: Genomic rearrangements involving *ERG* and *ETV1* (two members of the ETS family of transcription factors) are found in 50-70% of prostate carcinomas (PCa). The products of specific chimeric genes could be targeted therapeutically, but the nuclear localization of the aberrant ETS proteins makes them difficult therapy targets *in vivo*. This work aimed to evaluate whether *ERG* and *ETV1* regulate specific or shared target genes, as some may be more amenable to targeted therapy. METHODS: We performed differential expression analysis on nine normal prostate tissues and 50 PCa enriched for different ETS rearrangements using exon-level expression microarrays, followed by *in vitro* validation using cell line models. RESULTS: We found specific deregulation of 57 genes in *ERG*-positive PCa and 15 genes in *ETV1*-positive PCa, whereas deregulation of 27 genes was shared in both tumor subtypes. We further showed that the expression of seven tumor-associated ERG target genes (*PLA1A*, *CACNA1D*, *ATP8A2*, *HLA-DMB*, *PDE3B*, *TDRD1* and *TMBIM1*) and two tumor-associated *ETV1* target genes (*FKBP10* and *GLYATL2*) was significantly affected by specific ETS silencing in VCaP and LNCaP cell line models, respectively, whereas the expression of three shared candidate targets (*GRPR*, *KCNH8* and *TMEM45B*) was significantly affected by silencing of either ETS. Interestingly, we demonstrate that the expression of *TDRD1*, the top-most overexpressed gene of our list of *ERG*-specific targets, is inversely correlated with the methylation levels of a CpG island found at -66bp of the transcription start site in PCa and that *TDRD1* expression is regulated by direct binding of ERG to the CpG island in VCaP cells. DISCUSSION: We conclude that ETS transcription factors regulate specific and shared target genes and that *TDRD1*, *FKBP10* and *GRPR* are promising therapeutic targets and can serve as diagnostic markers for molecular subtypes of PCa harboring specific fusion gene rearrangements.

0060

LRP1B inhibits angiogenesis and metastatic potential of cancer cells by modulating the abundance of multiple factors in the extracellular environment

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The Low-density lipoprotein receptor-related protein 1B (LRP1B), encoding an endocytic LDL-family receptor, is among the most frequently deleted genes in human cancer. We have previously reported chromosomal, epigenetic and microRNA contributions in inactivation of this tumour suppressor gene in thyroid cancer. However, the overall implications of LRP1B endocytic activity in terms of the composition of the extracellular microenvironment are poorly studied. By employing antibody arrays, ELISA (and activity assays) on conditioned media from thyroid, bladder and melanoma cancer cell lines we originally show that LRP1B expression led (directly or indirectly) to changes the "extracellular proteome". Namely, a reduction in angiogenesis activators (PIGF, VEGF and VEGF-D), growth factors (SCF and Neuregulin 1), metalloproteinases (MMP2, MMP9 and TACE/ADAM17), decoy apoptotic receptors (TRAILR2), soluble adhesion molecules (NrCAM) and cytokines (TMEM, XEDAR) was seen upon LRP1B expression. We further validated by ELISA that TACE and PIGF are depleted in extracellular medium from LRP1B expressing cells. In vivo, LRP1B expression reduced the angiogenesis response and the metastatic potential of an anaplastic thyroid carcinoma cell line. These results of in vitro and in vivo studies afford elucidation into the mechanisms by which this endocytic receptor acts as a tumour suppressor, putting emphasis on the molecules that may be under LRP1B-mediated regulation in the tumour microenvironment.

0061

Differential Regulation of Basal and IL-7-induced PI3K/Akt/mTOR and Jak/STAT5 Signaling Distinguishes Pediatric from Adult ALL

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Acute lymphoblastic leukemia (ALL) represents about 80% of acute leukemias in children and 20% in adults, with strikingly poorer prognosis in adults. However, the biological determinants for such profound clinical difference remain largely unsettled. Here, we evaluated potential differences in the activation of PI3K/Akt/mTOR and JAK/STAT5 oncogenic pathways between pediatric and adult cases with B-cell precursor phenotype. ALL samples collected at diagnosis from children (n=19) and adults (n=24), and bone marrow samples from healthy donors (n=8), were analyzed by flow cytometry for phosphorylation of Akt (S473 and T308), S6 (S235/236) and STAT5 (Y694), and expression of PTEN and Akt, ex vivo or in interleukin-7 (IL-7)-stimulated leukemia cells. Adult ALL samples displayed significantly higher basal PI3K/Akt/mTOR pathway activation than healthy donors. In an apparent paradox, PI3K/Akt/mTOR pathway activation was frequently found together with overexpression of the tumor suppressor PTEN, which negatively regulates PI3K signaling. However, similar to our published data on childhood ALL of T-cell origin (Silva et al, JCI 2008), high PTEN levels were associated with decreased PTEN phosphatase activity in leukemia cells. Notably, the levels of PI3K/Akt/mTOR pathway activation were significantly lower in adult than in pediatric ALL cases. Moreover, in contrast to childhood samples, adults showed a significant positive correlation between Akt expression and Akt phosphorylation levels. These data suggest that basal PI3K-mediated signaling is differentially regulated in the two age groups. Strikingly, IL-7 promoted Akt, S6 and STAT5 phosphorylation in, respectively, 13, 13 and 50% of adults versus 25, 25, and 83% of pediatric cases, with an average fold induction in phospho-STAT5 of 1.378±0.1389 in adults versus 2.957±0.6766 in children (P=0.0312; 2-tailed Mann-Whitney), indicating increased responsiveness to this microenvironmental cytokine in pediatric ALL. Overall, we demonstrate that diagnostic pediatric and adult ALL samples differ significantly in their levels of constitutive PI3K/Akt/mTOR pathway hyperactivation and IL-7 signaling responsiveness.

0062

Using an *in vitro* model of Epithelial-Mesenchymal-Epithelial transitions to uncover novel biological mechanisms

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Epithelial-mesenchymal-transition (EMT) and mesenchymal-epithelial-transition (MET) are fundamental mechanisms controlling events such as embryogenesis and cancer. Cancerous cells undergoing EMT, exhibit a mesenchymal-like phenotype with increased invasion and apoptosis resistance, enabling detachment from the primary-environment. Nevertheless, establishment of cancerous cells at novel locations is only possible for cells that are successful in undergoing the reverse process MET. Although EMT inducers/cellular outcomes have been largely studied, molecular players of cells that revert through MET are far from being recognized. We produced a dynamic EMT/MET model to analyze the whole transcriptome variation and defining the biological-pathways that determine these transitions. We induced EMT/MET in a normal-cell-line by adding/removing TGF-β1. Total RNA extracted at distinct timepoints was subjected to whole transcriptome sequencing. Bioinformatic analysis was performed using in house pipelines and commercially-available-software. RNA expression alterations were verified by qRT-PCR, protein-expression by Western-Blot and immunofluorescence. Metabolism intermediates were measured using ELISA. We confirmed the occurrence of EMT/MET via analysis of the differential transcription of epithelial/mesenchymal markers. We have found thousands of genes differentially-expressed and our bioinformatics analysis correlated this differential-activation with uncounted cancer/metastasis-related pathways. Moreover, we have uncovered novel biological mechanisms underlying EMT/MET such as differential glycosylation of E-cadherin, alternative activation of metabolic pathways and novel epigenetic mechanisms, underlying activation/repression of recently-annotated genes. We were able to establish a dynamic *in vitro* model of EMT/MET, which allowed uncovering novel biological and genetic mechanisms using a non-biased genome wide approach.

0063

Somatic mutations and deletions of the E-cadherin gene predict poor survival of gastric cancer patients

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The prognosis of gastric cancer (GC) is poor and the molecular pathogenesis players vastly unknown. Surgery remains the primary option in GC treatment. The aim of this study was to investigate the impact of somatic *CDH1* alterations in prognosis and survival of GC patients. A series of sporadic and familial GC cases (diffuse and intestinal,n=246) were analyzed for somatic *CDH1* mutations, promoter-hypermethylation and loss of heterozigosity (LOH) by PCR-Sequencing. E-cadherin

expression was determined by immunohistochemistry. *CDH1* somatic-alterations were found in ~30% of all cases. Both histological types of sporadic GC displayed: LOH in 7.5%, mutations in 1.7% and hypermethylation in 18.4% of the cases. Primary tumors from Hereditary-Diffuse GC, lacking germline *CDH1* mutations, showed exclusively *CDH1* promoter-hypermethylation in 50% of the cases. Familial-Intestinal GC (FIGC) tumors showed LOH in 9.4% and hypermethylation in 17.0%. *CDH1* alterations did not associate with a particular pattern of E-cadherin expression. Importantly, the worst patient survival rate among all GC analyzed was seen in patients with tumors carrying *CDH1* structural alterations, preferentially those belonging to FIGC families. *CDH1* somatic alterations exist in all clinical settings and histotypes of GC and associate with different survival rates. Their screening at GC diagnosis may predict patient prognosis and is likely to improve GC patient management *(in press JCO)*.

0064

Breastfeeding and Helicobacter pylori infection: systematic review and meta-analysis

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Introduction: A previous systematic review including 16 studies showed a protective effect of breastfeeding in middle- and low-income settings. However, the assessment of this relation may be improved by stratified analyses taking into account more detailed and accurate definitions of the exposure. In last few years, a set of additional studies were published, making possible to update the existing evidence.
Objective: To quantify the association between breastfeeding and H. pylori infection, among children and adolescents, through systematic review and meta-analysis.
Methods: Pubmed was searched up to September 2012. Odds ratios (OR) and corresponding precision estimates, or the necessary information to calculate them, were extracted. The DerSimonian and Laird method was used to compute summary estimates and 95% confidence intervals (95%CI). Heterogeneity was quantified with the I2 statistic.
Results: Twenty six eligible studies were identified. Fifteen studies compared breastfed versus non-breastfed subjects; the summary OR was 0.73 (95%CI: 0.58 to 0.91, I2=46.4%). The summary OR was similar when considering only the results of the 6 studies providing estimates adjusted for potential confounders, and did not differ across the method used for the diagnosis of the infection, or between countries in which the prevalence of infection among the adult population is more or less than 50%. The nine studies that assessed the risk of H. pylori infection among subjects breastfed for ≥4 to 6 months versus <4 to 6 months yielded a summary OR of 0.89 (95%CI: 0.57 to 1.39, I2=70.9%). Only one study assessed the effect of exclusive breastfeeding (≥6 months versus <6 months: OR=0.91, 95%CI: 0.61-1.34).
Conclusion: Our results are compatible with a potential protective effect of breastfeeding. However, the assessment of broad categories of exposure, as well as the heterogeneity on the methods and reporting of results, precluded bold conclusions on this issue.

0065

Effects of new steroidal aromatase inhibitors on sensitive and resistant breast cancer cells

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Several therapeutic approaches are used in estrogen receptor positive (ER^+) breast cancers, being one of them the use of aromatase inhibitors (AIs). Although AIs demonstrate higher efficacy than tamoxifen, they can also exhibit *de novo* or acquired resistance after prolonged treatment. Recently, we have described the synthesis and biochemical evaluation of four new steroidal AIs, 3β-hydroxyandrost-4-en-17-one (**1**), androst-4-en-17-one (**12**), 4α,5α-epoxyandrostan-17-one (**13a**) and 5α-androst-2-en-17-one (**16**), obtained from modifications in the A-ring of the aromatase substrate, androstenedione. In this study, it was investigated the biological effects of these AIs in different breast cancer cell lines, an ER^+ aromatase-overexpressing human breast cancer cell line (MCF-7aro cells), an estrogen-receptor negative (ER^-) human breast cancer cell line (SK-BR-3 cells), and a late stage of acquired resistance cell line (LTEDaro cells). The effects of an autophagic inhibitor (3-methyladenine) plus AIs **1**, **12** or **13a** in LTEDaro cells were also studied to understand the involvement of autophagy in AI acquired resistance. As a reference aromatase inhibitor it was used exemestane. Our results showed that the new compounds inhibit MCF-7aro cells aromatase and decrease cell viability in a dose- and time-dependent manner. The new AI **1** is the most potent inhibitor, although the AI **12** demonstrates to be the most effective in decreasing cell viability. Besides, and in advantage over **exemestane**, AIs **12** and **13a** also reduced LTEDaro cells viability. The use of the autophagic inhibitor allowed AIs to diminish viability of LTEDaro cells, reverting the acquired resistance which suggests that autophagy is involved in resistance. Thus, inhibition of autophagy may play a role in sensitizing these hormone-resistant breast cancer cells to anti-estrogen therapies.

The authors are grateful to Fundação para a Ciência e Tecnologia (FCT) for the PhD grants attributed to Cristina Amaral and Carla Varela (SFRH/BD/48190/2008 and SFRH/BD/44872/2008, respectively) and for finantial support (PTDC/QUI-BIQ/120319/2010).

0066

Serrated polyposis with family history of polyps and/or colorectal cancer: a distinct clinical and molecular entity differing between the proximal and the distal colon

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***Introduction*:** Serrated polyposis (SPP) is characterized by the development of multiple colorectal serrated polyps and increased predisposition to colorectal cancer (CRC). We aimed to characterize at clinical and molecular level a cohort of SPP patients with or without family history of polyps (multiple or diagnosed at a young age) and/or CRC in first degree relatives (SPP-FHP/CRC). ***Methods*:** We analyzed 62 serrated or adenomatous lesions from 11 SPP-FHP/CRC families and 6 sporadic SPP patients for microsatellite instability (MSI), hypermethylation of *MGMT* and mismatch repair (MMR) genes, and somatic mutations in WNT and RAS/RAF genes. ***Results*:** SPP-FHP/CRC patients presented an older mean age at diagnosis (p=0.027), a more heterogeneous histological pattern of lesions (p=0.032) in comparison with sporadic SPP. Two forms of SPP-FHP/CRC appear to exist, according to the molecular alterations and to the preferential location of early lesions, proximal/whole colon and distal. Notably, MMR gene methylation was detected exclusively in the former [10/29 (34%) vs 0/18, p=0.0039]. Proximal/whole colon SPP-FHP/CRC presented also a higher frequency of MSI and WNT mutations [15/26 (58%) vs 2/15 (13%), p=0.006; 14/26 (54%) vs 4/20 (20%), p=0.02, respectively] but a lower frequency of *BRAF* mutations [12/20 (60%) vs 7/30 (23%), p=0.009], when compared with the distal form. Two groups of patients were identified in each form, whose lesions harboured preferentially *KRAS* or *BRAF* mutations, respectively. CRC was more frequent in proximal/whole colon SPP following a KRAS (alternate) pathway [4/4 vs 1/8 (12%), p=0.01]. ***Conclusions*:** We conclude that SPP-FHP/CRC appears to be a distinct clinical and molecular entity, presenting two different forms, proximal/whole colon and distal, the former with an early MMR deficiency. CRC risk appears to be higher in proximal/whole colon SPP-FHP/CRC following an alternate (KRAS) pathway.

0067

Evidence for reciprocal regulation between TAL1 and miRNAs in T-cell acute lymphoblastic leukemia

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The transcription factor TAL1 is progressively downregulated early in T-cell development and frequently overexpressed by unknown mechanisms in T-cell acute lymphoblastic leukemia (T-ALL). MicroRNAs can decrease the expression of numerous genes and have a clear impact in cancer. The interplay between TAL1 and particular miRNAs has not been explored, despite the speculation that TAL1 might be targeted by some miRNA families. In turn, although some TAL1 target genes have already been revealed, there are no studies on the regulation of miRNA genes by TAL1.
To identify putative miRNAs that target TAL1 we performed computational prediction of miRNAs that bind to TAL1 mRNA. To validate the candidate miRNA/TAL1 mRNA interaction we used a reporter plasmid with TAL1 3'UTR downstream of the luciferase ORF, together with enforced expression of the candidate miRNAs. We identified candidate miRNAs that knocked down luciferase expression in 30-50%. We are currently analyzing the impact of overexpression of these miRNAs on TAL1 expression in T-ALL cells.
To evaluate whether TAL1 transcriptionally regulates miRNA genes, we enforced the expression of TAL1 in one TAL1-negative T-ALL cell line and analyzed TAL1-dependent miRNA gene expression. A gene expression analysis for 372 human miRNA genes was performed and 11 microRNAs were selected for further validation. To this purpose we checked the impact of TAL1 ectopic expression or TAL1 siRNA-mediated knock down on miRNA gene expression by qRT-PCR. The results confirmed the data obtained in the screening for five miRNA genes, supporting the hypothesis that the expression of some of the selected miRNAs is regulated by TAL1. We are currently testing the ligation of TAL1 to a particular miRNA gene promoter region by chromatin-immunoprecipitation (ChIP).
Overall, our data suggest that TAL1 expression might be regulated by miRNAs and that, conversely, TAL1 may transcriptionally regulate the levels of defined miRNA genes.

0068

Regulation of the metabolic profile of cervical and breast cancer cells by hypoxia

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Background
One essential hallmark of tumour cells is the glycolytic phenotype, which can be an adaptive consequence of tumour hypoxic microenvironment. It has been described that monocarboxylate transporters (MCTs), by transporting lactate, are important in the maintenance of the glycolytic phenotype and intracellular pH homeostasis, contributing to acidic microenvironment. However, MCT regulation by hypoxia is not well understood, being even controversial,especially in what concerns MCT1.

Aims
We intended to characterize the expression of MCT1 and MCT4 and other glycolytic markers, under hypoxic conditions. We also aimed to evaluate the effect of hypoxia in cell metabolism,as well as determine the sensitivity to CHC (an MCT inhibitor),in human cervical and breast cell lines.

Material/Methods
Hypoxia was induced in human cervical and breast cancer cell lines, using a hypoxic chamber (<1% O2).Characterization of the expression of MCT1,MCT4,CD147,GLUT-1,CAIX and LDH was performed by immunocytochemistry and Western blot. The effect of hypoxia on cellular metabolism was assessed through quantification of glucose consumption and lactate production and the effect of CHC on cell total biomass through the Sulforhodamine B assay.

Results/Discussion
As expected, GLUT-1, CAIX and LDH increased with hypoxia.In general,MCT1 expression increased (e.g.:C33 and Hs578T cells) or its cell location was altered mainly to the plasma membrane (e.g.:SiHa cells).However, the increase of MCT4,which is described as highly induced under hypoxia,was not so evident.Curiously,the expression of the MCTs' chaperone (CD147) appeared to decrease in specific situations.As expected,the metabolic profile and sensitivity to CHC was also affected by hypoxic conditions.

Conclusion
In this study,we showed that hypoxia regulates the expression of MCTs,mainly MCT1,and is also responsible to sensitize some cells to MCT inhibition.Further studies will be needed,however these findings provide evidence for the regulation of MCTs by hypoxia and for the importance of MCT1 in glycolytic metabolism.
Acknowledgements
Part of this work was supported by the FCT grant ref. PTDC/SAU-FCF/104347/2008.

0069

The role of GRIM-19 in thyroid and kidney tumors

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Hürthle cell tumors (HCT) are a particular group of thyroid tumors composed by cells with oncocytic features (excessive mitochondria number with structural and/or functional alterations). The genetic alterations underlying the etiopathogenesis of oncocytic tumors remains to be clarified, although recent data suggest that mitochondrial DNA (mtDNA) complex I disruptive mutations may be involved(1) . GRIM-19 (Gene associated with Retinoid Interferon-induced Mortality - 19) is a novel tumor suppressor gene that is involved in interferon-ß (IFN-ß) and retinoic acid (RA)-induced cell death and is also a subunit of mitochondrial respiratory chain (MRC) complex I. STAT3 (Signal Transducer and Activator of Transcription 3) is one of the GRIM-19 interacting proteins, participating in IFN/RA-GRIM-19-induced cell death (2). Recently, GRIM-19 mutations and loss of proteins expression have been reported in several tumor types (3).
Our aim was to clarify the role of GRIM-19 and interacting STAT3 protein in tumorigenesis and in the development of the oncocytic phenotype.

We observed that GRIM-19 is downregulated in oncocytic (Hürthle) cell tumors of the thyroid, but not in non-oncocytic cell tumors, independently of the histotype and of the benign or malignant nature of the tumors. However, in kidney tumors, GRIM-19 is downregulated in all tumor histotypes. Furthermore, we did not observe any correlation between GRIM-19 expression and STAT3

activation. Preliminary data*, in vitro*, showed that GRIM-19 downregulation alters cell morphology and mitochondrial network. Additionally, a distinct metabolic profile was also observed.

This study shows GRIM-19 as the first nuclear DNA encoding a MRC complex I protein downregulated in HCT, supporting the assumption that mitochondrial complex I dysfunction is involved in the etiopathogenesis of oncocytic tumors, at least those occurring in the thyroid. Moreover, our data also suggests that GRIM-19 may have a broader role in kidney tumorigenesis playing a role in cell morphology and cell metabolic remodeling.

0070

A NOVEL ORTHOTOPIC METASTATIC CANCER MODEL OF COLORECTAL ADENOCARCINOMA WITH NON-INVASIVE NUCLEAR IMAGING TUMOR EVALUATION

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The orthotopic (ORT) model of implantation of human tumors in immunosuppressed mice has proved to be the most appropriate model compared to the subcutaneous models which doesn't characterize the tumor-microenvironment or expresses an invasive/metastatic phenotype.

Currently, the ORT model of colorectal adenocarcinoma (CRC) is in the cecum, however this is an unusual site in human CRC, therefore becomes necessary to establish two ORT models of CCR, in the cecum and left colon, through microsurgery with micro-injection of human CRC cell line WiDr in the mucous-fistula. The progression assessment was done resorting to nuclear medicine techniques, using ^{99m}Tc-MIBI and ^{18}F-FDG.

31 RNU rats underwent two surgical procedures (cecostomy and descending colostomy with distal mucous fistula) and injected with WiDr cells (10-14×10^6 cells/animal), after return to normal bowel function. Evaluation with ^{99m}Tc-MIBI and ^{18}F-FDG were performed after intravenous injection and acquired using a gamma-camera and a prototype ClearPEM, respectively.

For equal amount of cells inoculated in both models, Colostomy-induced model shown higher longevity and life quality, expressing slow progression of symptoms, contrasting to animals with cecostomies. Cecostomy-induced models expressed larger primary tumors, more invasive to surrounding tissues and organs, although with minor signs of distant metastases, opposing to colostomy-induced model, which although smaller primary tumors, evidence distant metastases (liver, lung and altered lymph mesenteric nodes). The radiopharmaceuticals shown tumor uptake in both models.

These preliminary data suggests that the colostomy model seems as the best model of CRC, the best in characterizing the tumor microenvironment, slow progression of symptoms, greater longevity and less diffuse tumors. Although good tumor tracers, the different characteristics of the radiopharmaceuticals used have clear advantages and disadvantages that can influence the proper interpretation.

This work was supported in part by FCT - Portugal and by QREN.

0071

Multidrug Resistance in Hepatocellular Carcinoma: in vitro studies with ^{18}F-FDG and ^{99m}Tc-MIBI

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Introduction and aim: Hepatocellular Carcinoma (HCC) is known to be highly resistant to chemotherapy, which is due in part to overexpression of multidrug resistance proteins (MDR). A common method to measure the function of these proteins involves the study of radiolabeled substrate ^{99m}Tc-MIBI uptake. Recent studies have demonstrated that ^{18}F-FDG uptake is associated with the expression of these proteins in HCC. This study aims to evaluate and compare the uptake and retention of ^{18}F-FDG and ^{99m}Tc-MIBI in two human HCC cell lines with different expression levels of p53 and to correlate with the expression of three MDR proteins (Pgp, MRP1 and LRP).

Materials and Methods: The human HCC cell lines used were HepG2(wp53) and HuH7(mp53). Cell suspensions were incubated with 2x10^{6}cells/ml with ^{18}F-FDG and ^{99m}Tc-MIBI (25µCi/ml). Samples of 200µl were collected at different periods of time which were centrifuged separating the supernatant from the *pellet*. The retention of ^{18}F-FDG and ^{99m}Tc-MIBI was obtained by incubating the cell suspension with radioisotope during 60 minutes. Thereafter, the cells were centrifuged and medium renewed. The following procedure was similar to the uptake studies. The proteins levels of Pgp, MRP1 and LRP were determined by flow cytometry.

Results: It was found that HuH7cell line is one that has higher levels of uptake and retention of ^{99m}Tc-MIBI and also ^{18}F-FDG. The HepG2 cell line has a lower uptake and retention and a higher expression of MRP1. The levels of Pgp and LRP expression are similar for both cell lines.

Conclusions: It is clear that there is an inverse relationship between MRP1 expressions and uptake and retention of ^{99m}Tc-MIBI and ^{18}F-FDG. The uptake and retention profiles for the two radiopharmaceuticals are similar, showing that the ^{18}F-FDG can be used to study the action of MDR proteins in HCC cells, presented as an alternative to ^{99m}Tc-MIBI.

0072

Oxysterols and Cancer. A Systematic Approach to Find New Cytotoxic Compounds and SAR Analysis.

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Oxysterols are oxidized derivatives of cholesterol formed via spontaneous and/or enzymatic oxidation processes and present in mammalian tissues at very low concentrations. They have been gaining much focus due to the diverse biological activities displayed in cell cultures. Oxysterols are intermediates of the biosynthesis of bile acids and steroid hormones and have been reported to interfere with the regulation of cholesterol homeostasis, inflammation, cell differentiation and proliferation. Moreover, they participate in the Hedgehog signaling pathways and are important regulators of lipid rafts.[1]

Our group has become interested in the antitumor activity of oxysterols. After studying the cytotoxicity of the endogenous rings A and B oxysterols present in the human organism, we have endeavored the synthesis of a large library of sterols by means of oxidative reactions at rings A and B, glycosylation at position C-3, as well as regioselective enzymatic acylations in the sugar moiety. The effects of the oxysterols obtained on the proliferation of cancer and non cancer human cell lines were investigated.

The oxysterols studied were cytotoxic in a dose-dependent manner and in the low micromolar range, against different human cells, being in general more potent towards cancer cells, as compared to non-cancer ones.
Although the effects were cell-dependent, structure-activity relationships, SAR, were set up and the key features for cytotoxicity were disclosed [2-4].

0073

mtDNA-induced OXPHOS dysfunction causes enhanced tumour growth and metastatic potential: a role for OXPHOS in regulating migration?

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Reprogramming of cellular metabolism is a hallmark of tumour cells and is thought to be required for tumour development by fulfilling the needs of a proliferating cell. Reports showing inactivating mutations in mitochondrial enzymes of the Krebs cycle and respiration underline a mechanism for the metabolic shift in tumour cells; therefore, dysfunction of mitochondrial oxidative phosphorylation (OXPHOS) seems to be a key feature which is often associated with mutations in OXPHOS-encoding mtDNA genes.
We have created a cell model for OXPHOS dysfunction based on the cybrid technology. From the same parental cell line (143B cells), we have successfully obtained three cell lines: one depleted of mtDNA (ρ0), a cybrid harbouring wt mtDNA and a cybrid harbouring a mutation in the mtDNA gene for tRNALeu(UUR). It was shown that this mtDNA mutation alters the cellular metabolism, leading to little or no OXPHOS activity and increasing glycolysis.
Our goal was to determine the effects of mtDNA-induced OXPHOS dysfunction in tumourigenesis. We saw no significant differences in the population doubling time between OXPHOS deficient and OXPHOS normal cell lines. However, upon injection in nude mice, the cells with OXPHOS deficiency gave rise to bigger tumours and tended to display more invasion and distant metastasis, suggesting that OXPHOS deficiency conferred increased metastatic potential in vivo. By timelapse microscopy, we observed an enhanced cellular migration in OXPHOS deficient cells than OXPHOS normal cells, a feature that appears to be dependent on increased production of ROS by OXPHOS deficient cells.
We have shown that mitochondrial dysfunction caused by mtDNA mutation is associated with an increased tumourigenic potential, particularly with higher migration/motility, which may be correlated with increased capability to invade new tissues and

metastasize. Upcoming results will reveal the mechanism linking OXPHOS dysfunction and migration capacity/metastic potential, which may be a putative target for cancer therapy.

0074

Immune Infiltration on feline endometrial adenocarcinomas

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In the queen, the uterus is the most common site of genital tract for the occurrence of tumors, though contributing to only 0.29% of all cancers diagnosed in these animals. Despite being considered a rare tumor in cats, our studies showed that feline endometrial carcinomas (FEA) occur more frequently than once thought. Aware of the importance played by the immune system, through a dynamic relation, in tumor development, this study aimed to assess the infiltration of immune cells in FEA lesions.

Ten samples of papillary serous FEA (classified upon hematoxylin-eosin sections) were used, along with ten samples each in estrogenic and progestagenic stages of the oestrous cycle (controls). Immunolabelling was performed using antibodies against macrophages, T cells and B cells (respectively: MAC 387, Ab-Serotec®, 1:100; CD3, Dako®, 1:50; and CD79, Cell Marque®). Immune cells were counted in two different layers on normal uterus and in tumors. Also, for tumor´ samples, the normal tissue surrounding the tumor has been evaluated.

The immune cells infiltrates differed among stages, which also differed towards the tumor samples. In the latter, immune cells infiltrate, independently of the cell type, were markedly increased in comparison to controls ($p<0.001$). Also, we also found that in some tumor samples (n=5:10) the tumor lesion co-existed with pyometra. In such situations, the increase in the immune cells infiltrates was even more marked, specially for B lymphocytes and macrophages.

0075

EXPRESSION OF CYTOKERATINS 7 AND 20 IN THE ENDOMETRIAL ADENOCARCINOMA OF THE QUEEN

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In Veterinary Pathology, endometrial adenocarcinomas are considered rare for all domestic species, except for cows and rabbits.

In human, endometrial adenocarcinomas usually conserve the same expression pattern for cytokeratins (CK) 7 and 20 than the normal endometrium: $CK7^{+}/CK20^{-}$. However, little is known about the expression of these CK in the queen.

For this study, 25 normal queen uteri were used to characterize the expression pattern of such proteins in the follicular (n=15) and secretory (n=10) phases of the oestrous cycle. Furthermore, the expression of CK7 and CK20 in 49 samples of endometrial adenocarcinoma was also analysed. All the samples came from the archives of the Laboratory of Histology and Pathological Anatomy of the University of Trás-os-Montes and Alto Douro.

The analysis of normal and neoplastic tissues was made through the streptavidin-biotin peroxidase immunohistochemistry, with primary monoclonal antibodies antibodies dilution of 1:100), and anti-CK20 (Eurodiagnostica®, dilution of 1:100). The intensity of the staining was evaluated through a 3-point score (weak, moderate and strong).

In normal uteri, the follicular phase presented a greater intensity of CK7 expression in comparison to the secretory phase while the expression of CK20 was moderate in both phases. The majority of the endometrial adenocarcinoma samples displayed a strong expression of CK7, whereas the CK20 expression was generally moderate. When comparing normal with neoplastic tissues, it is possible to perceive a decrease in the CK7 expression, while the expression of CK20 increases in the neoplastic samples.

This study contributed for a better characterization of feline endometrial adenocarcinomas, improving their correct diagnosis and understanding their clinical progression and prognosis.

0076

Survey of 548 oncogenic fusion transcripts in thyroid tumours supports the importance of the already established thyroid fusions genes.

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Neoplasms frequently present structural chromosomal aberrations that can lead to change in the level of expression of a protein or to the expression of an aberrant chimeric protein. In thyroid, *PAX8-PPARG* rearrangement is present in the neoplastic lesions that have a follicular architecture - follicular thyroid adenoma, follicular thyroid carcinoma and follicular variant of papillary thyroid carcinoma, while the presence of the *RET/PTC* rearrangements is largely restricted to papillary thyroid carcinoma.

The ability to detect fusion genes is relevant for a correct diagnosis and for therapy. We have developed a new fusion gene microarray-based approach for simultaneous analysis of all known and predicted fusion gene variants. We did a comprehensive screen for 548 known and putative fusion genes in 29 samples of thyroid tumours and one thyroid cancer cell line (TPC-1) using the fusion gene microarray. The TPC-1 cell line and 2 PTCs with known *CCDC6-RET* (alias *RET/PTC1*) fusion gene were used as positive controls. Validation of the array results was done by RT-PCR and FISH analysis.

Within the thyroid tumours tested, only well known, previously reported fusion genes in thyroid oncology were identified. Our results reinforce the pathogenic role played by *RET/PTC1*, *RET/PTC3* and *PAX8-PPARG* fusion genes in thyroid tumourigenesis.

0077

Interleukin-6 expression in bone marrow-derived cells regulates metastatic disease.

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Metastatic disease is the primary cause of cancer-related mortality, being responsible for more than 90% of cancer deaths. Improvements in cancer survival will only be tangible with a deeper knowledge and a better management of metastasis. In the past decade, there is a growing appreciation of the role of the cells comprising the cancer microenvironment in regulating metastatic progression. Identifying the crucial factors regulating these processes is the aim of this study. Clinically, elevated levels of the pro-inflammatory cytokine Interleukin-6 (IL-6) has been associated with advanced disease of different types. By using mice deficient in IL-6 we demonstrated that IL-6 knockout mice bearing orthotopically injected tumor cells (breast and melanoma) had a reduction in metastatic number and burden as compared to wild-type mice. Analysis of the pre-metastatic lungs and blood showed an increase of pSTAT3 activation in bone marrow derived cells (BMDC) during metastatic progression. Conditional overexpression of activated STAT3 demonstrated an increase in CD11b+Gr1+ cells in the lungs and blood that was abrogated after IL-6 knockout. STAT3 activation was also evident in the bone-marrow microenvironment, and this was associated with an increase in IL-6 expression in BMDC. Notably, a restoration of metastatic growth of tumors was observed in IL-6 knockout mice transplanted with wild-type bone marrow. Our results reinforce the concept of inflammation as a principal player in metastatic development and demonstrate an association between IL-6 expression in bone-marrow derived cells and metastatic disease.

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Breast tumor differentiation through Diffusional Kurtosis Imaging (DKI) in Magnetic Resonance Imaging

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Introduction
Diffusion is a physical property reflecting microscopic water molecules random motion, being influenced by surrounding cellular environment. Diffusion-weighted imaging(DWI) quantification in Magnetic Resonance Imaging(MRI) is used to differentiate tumors through Apparent Diffusion Coefficient(ADC). New models are being studied such as Diffusional Kurtosis Imaging(DKI) that, through Mean Kurtosis(MK) quantification parameter, measures non-Gaussianity of water motion in tissues, providing information regarding its complexity and structure. DKI has been mainly studied in brain and it can, potentially, add information to breast lesions characterization.

Purpose
Characterize and differentiate benign and malignant lesions and also histological lesion types through DKI.

Methods
20female patients were included with a mean age±standard deviation of 58.8±12.3years, considering 23breast tumors: 3benign(Fibroadenomas-FA) and 20malignant(16Invasive Ductal Carcinomas-IDC; 2Ductal Carcinomas *In Situ*-DCIS; 2Invasive Lobular Carcinomas-ILC).Informed consent was obtained from all participants. A DWI sequence was used on a 1.5T MRI scanner: Single-Shot Echo-Planar Imaging sequence, with 6b values in

3diffusion-sensitizing directions. Technical parameters were: TR/TE=12931/85ms; FOV=340x340mm^2; Matrix=228x226; thickness=3mm; bandwidth=1686.5Hz. Regions-of-Interest were placed on lesions and signal intensities(S) were measured. Mean Diffusivity(MD), which corresponds to ADC when MK=0, and MK were obtained by fitting next equation:
$S(b)= \{\eta^2+[S(0)\cdot(-b\cdot MD+1/6\cdot b^2\cdot MD^2\cdot MK)]^2\}^{1/2}$
Mean values were calculated and compared between lesions groups. Non-parametric statistics was used(α=0.05).

Results
MD and MK values for benign lesions were:(1.70±0.27)x10^{-3}mm^2/s and 0.50±0.44, respectively, and for malignant lesions:(1.36±0.37)x10^{-3}mm^2/s and 1.16±0.43. A significant difference between these groups(p=0.036) was observed in MK. MD and MK values for IDC were:(1.28±0.35)x10^{-3}mm^2/s and 1.28±0.36; DCIS:(1.80±0.28)x10^{-3}mm^2/s and 0.84±0.28; ILC:(1.60±0.61)x10^{-3}mm^2/s and 0.54±0.62. Between FA and IDC significant differences in MD(p=0.038) and MK(p=0.019) were observed.

Conclusion
The increased cellularity, typical in malignant tumors, could explain the decreased MD and increased MK, as it is when comparing IDC *vs.* FA lesions, where FA show increased MD and decreased MK. MK can potentially distinguish benign and malignant tumors, but also some of its subtypes.

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Nuclear medicine applied to prostate cancer: tumoral environment as a key factor

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Introduction: ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) application in nuclear oncology is based on upregulation of glucose transporters (GLUT) and glycolytic enzymes, associated with tumor hyperglycolysis. With regards to prostate cancer (PCa), it was not yet established a clear and direct relationship between these biochemical alterations and ^{18}F-FDG uptake. Recently, new oncological tracers for PCa have emerged, such as ^{18}F-Fluorocholine (^{18}F-CHO), which can replace or supplement the information given by ^{18}F-FDG.

Material and methods: Studies were performed in two PCa cell lines (ATCC): LNCaP (androgen/estrogen dependent) and PC3 (androgen/estrogen independent). Cell suspensions were incubated with ^{18}F-FDG or ^{18}F-CHO (25μCi/ml) and uptake studies were conducted under high (4.5g/L - HG) and low (1g/L - LG) glucose concentrations, as well as normoxia and hypoxia (2% of oxygen). GLUT protein expression was assessed by flow cytometry, as well as androgen receptor (AR) and Her2/neu expression.

Results: PC3 cell line has a higher uptake of ^{18}F-FDG over time in all conditions. When the same studies are performed in hypoxic environment, there is a slight increase in ^{18}F-FDG uptake. ^{18}F-CHO uptake is higher than ^{18}F-FDG, being the higher uptake registered when both cell lines are in HG. PC3 uptakes more ^{18}F-CHO than LNCaP cell line. GLUT-1 and -3 are major responsibles for glucose uptake in PCa cell lines under study, while GLUT-5 and GLUT-12 assume a role in mobilization of cytosolic glucose. Expression of AR in LNCaP is different according to level of glucose in culture medium. Her2 expression suggests a relation between its expression, AR expression and level of glucose in culture medium.

Conclusions: ^{18}F-CHO seems to be a better tracer then ^{18}F-FDG for PCa. Hypoxia did not appear to affect ^{18}F-FDG uptake, indicating that PCa does not resort to glycolysis to suppress energy needs. Her2/neu expression suggests a relation between its expression, AR expression and glucose concentration.

0080

Cell cycle deregulation and *TP53* and *RAS* mutations are major events in poorly differentiated and undifferentiated thyroid carcinomas

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Most thyroid carcinomas are well-differentiated and can be successfully treated. On the other hand, poorly differentiated (PDTC) and, particularly, anaplastic (undifferentiated) thyroid carcinomas (ATC) are among the most lethal malignancies, for which there is no effective treatment. Previously, we analysed PDTC genome-wide expression and found molecular signatures mainly related to cell proliferation, spindle assembly checkpoint and cell adhesion.

The aim of this study was to further elucidate the molecular pathways/alterations contributing to PDTC and ATC development. We profiled the global gene expression in 5 ATC, and analysed the mutational status of *RAS*, *BRAF*, *TP53*, *CTNNB1*, *PIK3CA* genes, of components involved in cell cycle control [*CDKN1A* ($p21^{CIP1}$); *CDKN1B* ($p27^{KIP1}$); *CDKN2A* ($p14^{ARF}$, $p16^{INK4A}$); *CDKN2B* ($p15^{INK4B}$); *CDKN2C* ($p18^{INK4C}$)], and in the epithelial-to-mesenchymal transition (EMT) regulation (*AXIN1*), in 23 PDTC and 26 ATC. *SNAI2* (SLUG) gene expression was also validated.

ATC molecular signatures were mainly related to cell cycle/proliferation, thyroid metabolism and cell junctions. In particular, gene expression profiling and quantitative PCR revealed up-regulation of the TGF-β target, *SNAI2* gene, which may be involved in the loss of epithelial/follicular morphology and increased mesenchymal properties.

PIK3CA and *CDKN2A* somatic mutations were detected in 15% of PDTC, but were less common in ATC (<5%). Furthermore, *BRAF* and *CTNNB1* mutations were detected in less than 5% of the tumours. Most somatic mutations were detected in *TP53* (22% of PDTC; 42% of ATC) or *RAS* (17% of PDTC; 31% of ATC). Mutations in *TP53* gene and mutations in *RAS* or *BRAF* genes showed evidence of mutual exclusivity (P=0.0193). Patients with *TP53* and/or *RAS* mutations had significant lower survival than *TP53* and *RAS*-negative patients (P<0.0338).

Overall, analysis of PDTC and ATC, confirmed the involvement of *TP53, RAS,* and *PIK3CA* genes, and revealed the contribution of genes, such as *CDKN2A*, that participate in cell cycle regulation.

Project supported by S.P.E.D.M.

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Characterization of MCT4, CD147, CD44 and GLUT1 immunohistochemical expression in Hepatic Metastases of Colorectal Cancer

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Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of death worldwide. During the course of this disease, 50% of the patients develop hepatic metastization and more than 66% have hepatic disease at the moment of death. Most patients die due to disease dissemination and so it is essential to understand the molecular events involved in tumour progression. Solid tumours depend mostly on glycolysis to fuel their elevated rates of proliferation, thus leading to an overload of lactic acid, which must be exported to the extracellular milieu, through the monocarboxylate transporters (MCTs). The expression of these transporters in the cell membrane requires the presence of chaperones, namely CD147 and CD44.

The objective of this study was to assess the immunohistochemical expression of MCT4, GLUT1, CD147 e CD44 in hepatic metastasis and adjacent healthy tissue of 45 patients with histologic diagnosis colorectal cancer with hepatic metastasis. We also tried to evaluate the association between MCTs and the remaining proteins and establish possible correlations between the tumour tissue expression and the clinicopathological data.

Our results showed that MCT4, its putative chaperone (CD147 and CD44) and the glucose transporter GLUT1 are overexpressed in the cell membrane of tumour samples. Furthermore, we observed that MCT4 membrane expression was associated with the remaining proteins associated with the glycolytic phenotype. Concerning the correlations between tumour tissue expression and the clinicopathological data, it was observed a statistically significant correlation between CD147 expression and venous involvement.

This is the first study done in hepatic metastases of CRC assessing the immunohistochemical expression of the transporters MCT4 and GLUT1 and the glycoproteins CD147 and CD44. Our results confirm their importance in tumour progression and the metastization process.

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Ascorbic acid has a cytotoxic effect in a melanoma cell line

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Introduction: Malignant melanoma is a type of skin cancer that in the metastatic stage doesn't respond to current therapies. Ascorbic acid (AA), the reduced form of vitamin C, may have a pro-oxidant activity. The increased production of hydrogen peroxide, coupled with the breakdown of the activity of antioxidant enzymes and the presence of transition metals in cancer cells, may

result in the selective cytotoxicity of vitamin C and the subsequent revelation of its therapeutic potential. The aim of this study is to evaluate, *in vitro* and *in vivo*, the cytotoxic effect of AA in a melanocytic melanoma cell line.

Methods: A-375 cells were incubated with different concentrations of AA (0,25 - 10mM). The half maximal inhibitory concentration (IC50) was calculated after 24, 48, 72 and 96 hours by the SRB assay. In order to evaluate cell survival, clonogenic assays were performed. Flow cytometry was performed to determine cell viability and death, ROS production, alteration of mitochondrial membrane potential and cell cycle. In order to verify *in vivo* the evolution of tumor growth, Balb/c nu/nu xenografts were daily submitted to intraperitoneal therapy with AA.

Results: AA induces a decrease in cell proliferation and survival in a dose dependent manner, being the IC50 less than 1,4mM. AA also induces a cytotoxic effect when cells are treated with 10mM of AA, being with this dose also observed an increase in intracellular superoxide radical and cell cycle arrest. Our studies also show a decrease in the ratio aggregates/monomers in a dose dependent way. The *in vivo* studies suggest that AA administered daily at 150mg/kg inhibits tumor growth.

Conclusion: AA induces a decrease in cell survival, proliferation and viability in A-375 cells, that is supported by the *in vivo* studies. These results suggest that AA may have a potential anti-cancer effect in melanoma cell lines.

0083

AntimiRs and miR-mimetics in the identification of miR function

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MicroRNAs or miRs are modulators of gene expression, which have recently revolutionized the traditional understanding of gene expression regulation. These small double-stranded RNA molecules have the capacity to control crucial functions in the cell. More importantly, as they may bind simultaneously several mRNAs, they can control the expression of various proteins which have different functions in the cell and therefore influence simultaneously several intracellular pathways [1]. Even though miRs have an enormous potential to control cellular fate, there is little understanding of how they select the mRNAs to which they bind and of how that may depend on the particular cellular context. In addition, miRs have been found in microvesicles, providing evidence for their capacity to control not only intracellular but also intercellular gene regulation.

The use of antimiRs (of single-stranded DNA antisense oligonucleotides) and miR-mimetics (of double-stranded RNA sequences) provide laboratorial tools to understand the phenotype of individual cellular miRs. We will present data that provides evidence that specific downregulation of miRs expression with antimiRs or the upregulation of miR expression with miR mimetics is possible in leukaemia cell lines. Downregulation of miR-21 was possible with antimiRs and upregulation of miR-128-1 was possible with miR-mimetics. Confirmation of miR levels was carried out by real-time qPCR and the analysis of cellular phenotype was possible with various assays such as BrdU for analysis of cellular proliferation, flow cytometry for analysis of cell cycle profile, TUNEL and Annexin V for analysis of apoptosis and confocal microscopy and Western Blot for analysis of authophagic cell death. Sensitization to doxorubicin and etoposide was possible by concomitant treatment with these drugs and the antimiR-21 or the miR-128-1 mimetic.

This work demonstrates the power of antimiRs and miR-mimetics in the identification of miR-21 and miR-128-1 function, in leukaemia cell lines.

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Acknowledgements: Fundação Calouste Gulbenkian for financial support to MHV and JEG. FCT for PhD and Post-Doc scholarships to HS (SFRH/BD/47428/2008) and RTL (SFRH/BPD/68787/2011). GMA supported by FCT and the European Social Fund.

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Sponsorship

We wish to express our appreciation for the significant support provided by the sponsors of this symposium.

Their interest and enthusiasm has enabled the meeting to take place.

FUNDAÇÃO ENG. ANTÓNIO DE ALMEIDA

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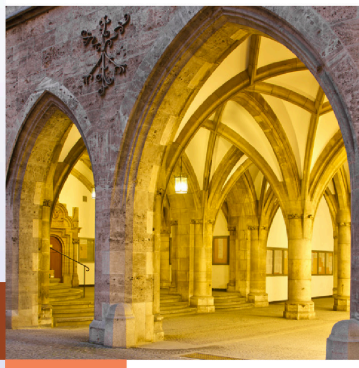

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