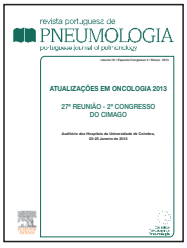
Rev Port Pneumol. 2013;**19(Esp Cong 2):**9-36

revista portuguesa de
PNEUMOLOGIA
portuguese journal of pulmonology
www.revportpneumol.org



POSTER PRESENTATIONS

Atualizações em Oncologia 2013

27ª Reunião - 2º Congresso do CIMAGO

Auditório dos Hospitais da Universidade de Coimbra, 23-25 Janeiro de 2013

The abstracts are the author's responsibility.

CANCER AND ENVIRONMENT

P1. DETERMINING GENETIC SUSCEPTIBILITY TO ISONIAZID-INDUCED HEPATITIS

M.C. Alcobia, M.T. Carvalho, L. Mesquita, M. Villar, C.R. Cordeiro, C. Fontes Ribeiro, H.C. Silva

Center of Pneumology, FMUC; Medical Genetics Unit, FMUC; CDP, Coimbra; CDP, Venda Nova; Department of Pharmacology and Therapeutics, FMUC; CIMAGO.

Introduction: Isoniazid (INH) is the most effective and widely used drug for treatment of tuberculosis. The identification of risk genotypes offers the possibility of individualization of INH therapy. INH plasma concentrations are highly dependent on metabolism via acetylation by the polymorphic enzyme N-acetyltransferase 2, encoded by *NAT2* gene. *NAT2* genotyping, allows the classification of individuals as "fast acetylators" (FA), intermediate acetylators (IA) or "slow acetylators" (SA), the latter with high risk for hepatotoxicity. Polymorphisms in genes encoding other enzymes, like *CYP2E1*, *GSTM1* or *GSTT1* are also involved in INH metabolism, and in the case of GSTs, a role in the hepatocyte response to chemical-induced stress cannot be ignored. Genetic variants decreasing proteins involved in bile salt transport may also be implicated, as is the case of V444A missense polymorphism in *ABCB11* gene.
Objectives: The aim of this study was to evaluate the contribution of polymorphism of genes *NAT2*, *CYP2E1*, *GSTM1*, *GSTT1* and *ABCB11*, to the susceptibility to INH-induced hepatitis.
Methods: A total of 109 treated tuberculosis patients from CDP of Coimbra and Venda-Nova were genotyped. Ten polymorphisms of *NAT2* were genotyped by sequencing; polymorphisms in *CYP2E1* (rs6413432 e rs2031920) and *ABCB11* (rs2287622) were analyzed by PCR-RFLP assay and homozygous for *GSTM1* and *GSTT1* deletions (*GSTM1*0/*0* and *GSTT1*0*0*) were identified using a PCR multiplex assay.
Results: Clinical variables such as age, alcoholic habits or previous hepatitis, were not associated with the occurrence of INH-induced hepatitis. Slow acetylators (52.3%) identified by NAT2 genotyping were significantly more prone to develop hepatotoxicity (p = 0.01; OR = 3; 95% CI = 1.23-7.35). Polymorphisms of *CYP2E1*, *GSTM1* and *GSTT* were not associated with the phenotype. For *ABCB11* polymorphism, homozygous for variant Ala had increased risk of developing hepatotoxicity (OR = 2.1; 95%CI = 0.9-5), though not reaching statistical significance. This effect was more evident for females than for males (OR = 2.19; CI = 0.45-10.58). Population was in Hardy-Weinberg equilibrium for all polymorphism.
Conclusion: INH-induced hepatitis is an unpredictable complication of tuberculosis treatment. *NAT2* genotyping is useful to establish a personalized dosage of INH and also may help to define more susceptible patients. Polymorphism in *ABCB11* gene deserves further investigation, especially among women.

P2. NUTRITION IN THE ELDERLY

F.M.A. Figueiredo, J.M.M. Marques, J.P.R. Tavares, P.L. Ferreira, M. Dourado

Faculty of Medicine, University of Coimbra; Hospital and University Center of Coimbra; Faculty of Economics-University of Coimbra; Continuing and Palliative Care Post- graduate Course - Faculty of Medicine - University of Coimbra.

Introduction: Aging is a complex process in which various factors, among which the nutritional balance, influence and are central to the maintenance of the quality of life. Therefore, in order to optimize the aging process, ensuring the best conditions of health and to prevent or control the main diseases in the elderly, there is a need for an adequate and balanced nutrition.
Objective: To study the nutritional status of elderly residents in a Private Social Solidarity Institution.
Methods: A non-probability sampling of convenience was included in the study, n = 82, 24 male and 58 female. The studied population was previously submitted to cognitive assessment via Mini Mental State. Ethical standards inherent in the Helsinki Declaration have been respected. Voluntary participation was required. Anonymity,

confidentiality was guaranteed. An informed consent was obtained. The Mini nutritional assessment MNA® range of Nestlé Nutrition Institute, a questionnaire that comprises eighteen items grouped into four categories, was applied. Alimentary behaviour as well as quality, quantity and diversity of rations were analysed, in order to diagnose the nutritional status. Data were statistically processed by SPSS (Statistical Package for the Social Science) program, by using the version 16.0 of 2007. Regarding the studied population, our results show that: For food intake, 4,9% had a severe decline;11% decreased moderately; 84.1% did not show any decrease; For weight loss, 1.2% has lost more than 3 kg; 13.4% lost between 1-3 kg; 46.3% has not lost weight; 39% did not respond; Body mass index distribution, 23.2% have less than 19; 11% between 19-21; 20.7% between 21-23; 45.1% greater than 23. The final score of nutritional status assessment showed that 26.8% have a normal nutritional condition (24-30 points); 48.8% have risk of malnutrition (17-23.5 points); 24.4% are malnourished (minus 17 points).
Conclusion: This study allowed us to analyze the nutritional status of the elderly with their particular sensibilities and, in the framework of a multidisciplinary work, to identify possible risk of malnutrition, assigning them custom strategies or even establish a future action protocol. We conclude that malnutrition risk is prevalent suggesting that attention must be given to the nutritional needs of elderly population. In this way the implementation of correct nutritional strategies could be helpful in reducing risk of diseases associated with malnutrition in the elderly.

P3. THE PRIMARY BILE SALT CHENODEOXYCHOLIC ACID INHIBITS BUTYRATE UPTAKE IN INTESTINAL EPITHELIAL CELLS

P. Gonçalves, T.A. Catarino, I. Gregório, F. Martel

Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Porto.

Introduction: Colorectal cancer (CRC) is one of the most common cancers worldwide. A diet rich in dietary fiber is associated with a reduced risk of CRC. Butyrate (BT) is one of the main end products of anaerobic bacterial fermentation of dietary fiber in the human colon. BT is an important metabolic substrate in normal colonic epithelial cells and has also important homeostatic functions at this level, including the ability to prevent/inhibit colon carcinogenesis. BT is transported into colonic epithelial cells by two specific carrier-mediated transport systems, the H^+-coupled monocarboxylate transporter 1 (MCT1) and the Na^+-coupled monocarboxylate cotransporter (SMCT1). Epidemiological and experimental studies suggest that bile acids may play a role in CRC etiology. So, our aim was to characterize the effect of the primary bile acid chenodeoxycholic acid (CDCA) upon [^{14}C]-BT uptake in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines.
Methods: [^{14}C]-BT uptake by Caco-2 and IEC-6 cell lines was quantified by liquid scintillometry; the expression of MCT1 and SMCT1 was quantified by qRT-PCR; the effects of BT and CDCA on cell viability, proliferation and differentiation were quantified by the lactate dehydrogenase assay, sulforhodamine B assay and alkaline phosphatase activity assay, respectively.
Results: A 2-day exposure to CDCA markedly and concentration-dependently inhibited [^{14}C]-BT uptake by IEC-6 cells (IC_{50} = 120 μM), and, less potently, by Caco-2 cells (IC_{50} = 402 μM). The inhibitory effect of CDCA upon [^{14}C]-BT uptake was not associated with a decrease in cell proliferation or viability. In IEC-6 cells: (1) uptake of [^{14}C]-BT involves both a high-affinity (SMCT1) and a low-affinity (MCT1) transporter, and CDCA acted as a competitive inhibitor of the high-affinity transporter; (2) CDCA inhibited both MCT1 and SMCT1-mediated uptake of [^{14}C]-BT; (3) CDCA significantly increased the mRNA expression level of SMCT1; (4) at the molecular level, the inhibition of [^{14}C]-BT uptake by CDCA was dependent on Ca^{2+}/calmodulin (CaM), mitogen-activated protein kinases (ERK1/2 and p38 pathways), and protein kinase C activation, and reduced by a reactive oxygen species scavenger. Finally, BT (5 mM) decreased IEC-6 cell viability and increased IEC-6 cell differentiation, and CDCA (100 μM) reduced this effect.
Conclusion: CDCA is an effective inhibitor of [^{14}C]-BT uptake in tumoral and non-tumoral intestinal epithelial cells, through inhibition of both MCT1 and SMCT1-mediated transport. Given the role played by BT in the intestine, this mechanism may contribute to the procarcinogenic effect of CDCA at this level.
Supported by FCT and COMPETE, QREN and FEDER (PTDC/SAU-FCF/67805/2006).

P4. CANCER STEM CELLS: THE DARK PASSENGER

J.P. Fonseca, I. Carreira, A. Mascarenhas, L. Carvalho, A.F. Ladeirinha, C.F.D. Rodrigues, M.C. Alpoim

Life Sciences Departament, University of Coimbra, Coimbra; CIMAGO, University of Coimbra, Coimbra; Faculty of Medicine, University of Coimbra, Coimbra; CNC, University of Coimbra, Coimbra; PDBEB Programme, CNC, University of Coimbra, Coimbra.

Introduction: Cancer is a heterogeneous entity that still represents one of the most common human diseases. In the last few years, a small population of cells called cancer stem cells (CSCs) has been identified in a wide range of tumors and blamed for their recurrence and resistance to the conventional therapies. Our lab has successfully established an adequate *in vitro* cellular model for the study of hexavalent chromium [Cr(VI)]-induced lung carcinogenesis. To this end, a non-tumorigenic human bronchial epithelial cell line (BEAS-2B) was exposed to sub-cytotoxic concentrations of Cr(VI), resulting in the establishment of the malignant RenG2 system. Two additional derivative cellular systems (DRenG2 and DDRenG2) were subsequently implemented out of tumors induced in mice. When assessing the role of CSCs in Cr(VI)-induced malignant transformation of BEAS-2B cells, we observed that the derivation process in mice resulted in the emergence of a CSCs subpopulation within the derivative systems, through a paracrine-induced dedifferentiation process of RenG2 cells.
Methods: The proliferation rate of Cont1 (the RenG2 non-malignant control), RenG2, DRenG2 and DDRenG2 cell lines, as well as of their normal precursor BEAS-2B, was evaluated using the trypan blue method. Further characterization was attained using karyotypic analysis and immunocytochemistry (MNF116, Vimentin and Oct3/4). Finally, chemoresistance to gemcitabine, cisplatin and methotrexate was accessed by the MTT assay and correlated with the presence/absence of the efflux pump P-Glycoprotein (P-gp) by immunocytochemistry.
Results: The attained results demonstrated that the derivatives, which are the more malignant and proliferative cell lines and the ones with a subpopulation of CSCs, showed marked structural and numeric alterations when compared to BEAS-2B cells. The DRenG2 predominant structural alterations were the $7q^-$ and the $iso9q^+$, while in the DDRenG2 it were the t(7:14) and the $17q^+$. In contrast to BEAS-2B, and similarly to RenG2, both derivatives' average ploidy was 75/76 chromosomes. Immunocytochemistry analysis revealed that all cell lines were mesenchymal-like (Vimentin-positive) and that they all have an epithelial origin (MNF166-positive). As expected the more malignant cell lines were significantly more resistant to the drugs under study, despite their P-gp negative phenotype.
Conclusion: The higher chemoresistance of the derivative cell lines correlates with the observation that tumors with CSCs subpopulations are highly resistance. Nevertheless, their negative staining for P-gp indicates that mechanisms other than extrusion transporters are responsible for the observed resistance.

P5. METABOLIC REPROGRAMMING ELICITED BY CARCINOGENIC CHROMIUM(VI) IN HUMAN BRONCHIAL EPITHELIAL CELLS

J.F. Cerveira, M. Sánchez-Aragó, A.M. Urbano, J.M. Cuezva

Unidade de Química-Física Molecular and Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra; Centro de Biología Molecular Severo Ochoa, CIBERER, Instituto de Investigación Hospital 12 de Octubre, Universidad Autónoma de Madrid; CIMAGO, Universidade de Coimbra, Coimbra.

Introduction: Investigations into the impact of hexavalent chromium [Cr(VI); chromate] on energy metabolism have been rather sparse. Still, it is noteworthy that all studies on Cr(VI) impact on respiration revealed an inhibitory action, an alteration compatible with the Warburg effect. In this study, we sought to extend our investigations into the bioenergetic changes and underlying mechanisms that occur when human bronchial epithelial cells, the main *in vivo* targets of chromate, are exposed to this carcinogen. To this end, BEAS-2B cells were exposed, for 48 h, to 1 μM of Cr(VI) and the effects on several critical bioenergetic parameters were determined under different experimental conditions. Protein levels of the catalytic subunit of the mitochondrial H^+-ATP synthase (β-F1-ATPase) and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were also determined.
Methods: OCRs were measured using the Seahorse XF24 Extracellular Flux Analyzer. Lactate levels were determined enzymatically. Intracellular ATP levels were determined using a commercial bioluminescence kit. Primary mouse monoclonal antibodies anti-Hsp60 and anti-GAPDH, and an anti-β-F1-ATPase polyclonal rabbit antibody were used in Western blot analysis. Results were statistically analysed with the unpaired Student's t test.
Results: We found that this short-term exposure to a subcytotoxic Cr(VI) concentration stimulated "aerobic glycolysis" under basal conditions, in the presence of oligomycin and, particularly, in the presence of an uncoupler. On the contrary, OCRs were lower in Cr(VI)-treated cells. Again, this effect became particularly evident under uncoupling conditions. These metabolic changes resulted in slightly decreased ATP levels. Consistent with augmented glycolytic fluxes, the expression of GAPDH was significantly increased in Cr(VI)-treated cells. The observed down-regulation of the β-F1-ATPase may account for the decreased respiratory capacity exhibited by the Cr(VI)-treated cells. Finally, levels of oxidative stress were augmented when Cr(VI)-treated cells were subsequently treated with oligomycin.
Conclusion: Our results confirmed that Cr(VI) exerts an inhibitory action on mammalian cell respiration. Concomintantly, it stimulated "aerobic glycolysis". The observed changes in the β-F1-ATPase/GAPDH protein ratio (viewed as a cellular bioenergetic index) upon Cr(VI) treatment may have contributed to the observed phenotypic changes.
Work supported by grants MICINN-BFU2010-18903, CIMAGO 26/07 and PEst-OE/QUI/UI0070/2011.

P6. CONTRIBUTION OF AN ALTERED MICROENVIRONMENT TO EPITHELIAL CELLS' TRANSFORMATION

M.M. Val, L.A. Mendes, M. Chanson, I. Carreira, L. Carvalho, A.F. Ladeirinha, C.F.D. Rodrigues, M.C. Alpoim

Life Sciences Department, University of Coimbra, Coimbra; CIMAGO, University of Coimbra, Coimbra; Laboratory of Clinical Investigation III, Department of Pediatrics, Geneva University Hospitals and University of Geneva, Geneva; Faculty of Medicine, University of Coimbra, Coimbra; CNC, University of Coimbra, Coimbra; PDBEB Programme, CNC, University of Coimbra, Coimbra.

Introduction: Cellular senescence (CS), defined by permanent cell cycle arrest, is a physiological process that serves as a powerful barrier for tumorigenesis in epithelial cells. However, senescent cells can concomitantly be deleterious for the tissue microenvironment. Such is the case of senescent fibroblasts that release several pro-tumorigenic factors, referred to as the senescence-associated secretory phenotype (SASP), that act in the nearby epithelial cells fueling their malignant transformation. Senescent stromal fibroblasts may also confer invasive and migratory phenotypes by promoting epithelial to mesenchymal transition (EMT) on the surrounding epithelial cells.
Methods: Aiming to understand the evolutionary dynamics generated by the interaction between human bronchial epithelial cells (BEAS-2B cells) and senescent human bronchial fibroblasts, co-cultures of BEAS-2B cells and either normal or senescent E2A fibroblasts were established and monitored for 4 weeks. Senescence was initially attained by exposing E2A fibroblasts for 4 weeks to 0.25 or 0.5 μM hexavalent chromium [Cr(VI)], a well know carcinogenic agent whose role on the etiology and progression of lung cancer is still unclear. Afterwards, a transwell system was used to co-culture the fibroblasts with BEAS-2B cells. Co-cultures were maintained using either normal, 0.25 μM-induced or 0.5 μM-induced senescent fibroblasts, and BEAS-2B cultivated in the absence or presence of the same Cr(VI) concentrations. Along culture, both cell types were screened for changes in their morphology and subsequently for alterations in the expression of relevant biomarkers of epithelial and mesenchymal phenotypes.
Results: Under the pressure of 0.25 μM Cr(VI), the senescent fibroblasts drove 0.25 μM Cr(VI)-exposed epithelial cells to acquire characteristics of invasive squamous cell carcinoma. Unexpectedly, 0.5 μM Cr(VI) concentration reverted fibroblasts' senescent phenotype while inducing features of transformed cells on the BEAS-2B cells. Also, under the pressure of 0.5 μM Cr(VI) senescent fibroblasts lead untreated epithelial cells to acquire a more differentiated phenotype. Finally, features of a pleomorphic carcinoma acquired via an EMT-like process were displayed by epithelial cells exposed to 0.5 μM Cr(VI) in the presence of non-senescent fibroblasts.
Conclusion: Our results suggest that epithelial cells and senescent stromal fibroblasts establish a crosstalk mediated by paracrine factors secreted by both cell types. This intercompartment communication predisposes epithelial cells for malignant transformation, facilitating the initial steps of the tumorigenic process. Later on, these same communication pathways may be used to sustain tumor growth and invasion.

GENETICS AND GENOMICS

P7. CHROMOSOME 1P36 REARRANGEMENTS: HOW COMPLEX IMBALANCES CAN BE DISCLOSED BY ARRAY-CGH

S.I. Ferreira, A. Jardim, A. Mascarenhas, N. Lavoura, E. Matoso, L. Ramos, J.B. Melo, I.M. Carreira

Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra; Laboratório de Citogenética, Hospital Pediátrico Carmona da Mota, CHUC-Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia; Serviço de Genética, Hospital Pediátrico Carmona da Mota, CHUC-Coimbra.

Introduction: Monosomy 1p36 deletion syndrome (OMIM 607872) is perhaps the most commonly observed terminal deletion in the population with an incidence of 1 in 5,000 live births. All patients present mental retardation and developmental delay, a characteristic facial phenotype and other phenotypic features.

Although the majority of cases present pure terminal deletions with variable breakpoints, complex rearrangements, consisting of either multiple deletions or terminal deletions occurring simultaneously with duplications or even triplications, also occur. The occurrence of complex rearrangements origins a variable phenotype whose clinical manifestations depend on the overexpression or haploinsufficiency of the genes involved in the imbalances.
Methods: We performed oligoarray-CGH using an Agilent 180K whole genome array in a 8 year old male patient with severe mental retardation, motor delay, hypotonia, absence of speech, feeding difficulties, behavioral anomalies, epilepsy, strabismus and other dysmorphic features.
Results: We report a patient with a complex *de novo* rearrangement at 1p36.33p36.21 involving a major terminal duplication of 2.4 Mb and a minor one, together with a major deletion with 6.7Mb, and a minor deletion with 623 Kb. The most striking characteristic of this patient is that his terminal imbalance is a duplication rather a deletion.
Conclusion: Of the 79 genes included in the 2.4Mb duplicated region of the present case, the *MMP23* genes are involved in bone remodeling and breakdown of extracellular matrix. Overexpression of these genes results in craniosynostosis, consistent with our patients phenotype. *SKI* gene deletion is involved in facial clefting anomalies observed, but our patient presents depressed nasal bridge and eye anomalies and has a duplication of the gene. The 6.7Mb deleted region encompasses 87 genes, one of which is *KCNAB2*, a candidate gene for the occurrence of epilepsy in patients with 1p36 deletion, like the reported case. These patients presents many features associated with the 1p36 deletion, besides having the commonly deleted region in duplication. This raises the question discussed by Redon of the position effect. Despite having a terminally duplicated segment, the expression of the duplicated genes might be altered by the proximity of the interstitial deletion. One example is the manifestation of some of the features associated with the deletion of *SKI* gene, which in the reported patient is duplicated. All together, these data reinforce this position effect idea, help to understand how patients with variable deletion sizes and different rearrangements have similar phenotypes and alert for the necessity to characterize 1p36 rearrangements with high resolution techniques.

P8. GENOMIC IMBALANCES AT CHROMOSOME 16P11.2 ASSOCIATED WITH MIRROR CONTRASTING PHENOTYPES

J.B. Melo, S.I. Ferreira, L.M. Pires, A. Jardim, M.C. Pinto, R. Monteiro, S. Neto, A. Luz, C. Nunes, F. Ramos, J. Sá, L. Ramos, J. Saraiva, I.M. Carreira

Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra; Serviço de Pediatria do Centro Hospitalar Leiria-Pombal; Serviço de Genética, Hospital Pediátrico Carmona da Mota, CHUC-Coimbra.

Introduction: Microarray-based comparative genomic hybridization (array-CGH) has revolutionized the genetic diagnostic of patients with global developmental delay (GDD), intellectual disability (ID) with or without dysmorphisms, multiple congenital anomalies (MCA) and autism spectrum disorders (ASD). The diagnostic yield has improved and new syndromes have been described. One of those syndromes is the occurrence of a Copy Number Variant (CNV) both in deletion and in duplication at chromosome 16p11.2. The clinical spectrum is variable and the manifestations typically associated are: language delay, ID, GDD, ASD, dysmorphic features, abnormal head size and behavioral problems.
Methods: We studied a cohort of 500 patients with GD, ID, MCA and ASD using an Agilent 180K whole genome oligonucleotide array-CGH.
Results: We detected five patients with imbalances at 16p11.2, three males and two females, being three deletions (one *de novo*, one paternal and one unknown) and two duplications (one *de novo* and one unknown). The phenotype of the patients is variable and comprises cognitive impairment, GDD, autism, dysmorphisms in some patients and behavioral problems. The observed CNVs range in size from 445 Kb to 545 Kb, with 28 genes involved, except for a patient with a 445 Kb duplication of unknown origin, where only 26 genes are included. In this case the proximal breakpoint is different and more distal. The majority of the previously reported deletions were *de novo*, and we report a paternally inherited deletion from a phenotypically normal father.
Conclusion: Recently, mirror contrasting phenotypes have been associated with gene dosage at the chromosome 16p11.2 locus. The phenotypic variability and the existence of incomplete penetrance for 16p11.2 imbalances represent a challenge for the clinical interpretation of the impact of these CNVs in the patients' phenotype and for the genetic counseling of carrier families.

P9. GENETIC TESTING IN HYPERTROPHIC CARDIOMYOPHATY AND SUDDEN CARDIAC DEATH: THE IMPACT OF THE NEW TECHNOLOGIES

A. Mascarenhas, A. Santos, M.C. Pinto, C. Pais, M. Venâncio, S. Santos, A.R. Fernandes, C. Monteiro, F. Corte-Real, I.M. Carreira

Laboratório de Citogenética, Faculdade de Medicina da Universidade de Coimbra, Coimbra; Laboratório de Genética Molecular de Cardiopatias, Coimbra; Serviço de Genética, Hospital Pediátrico Carmona da Mota, CHUC, Coimbra; Universidade Lusófona de Humanidades e Tecnologias, Lisboa; CQE, Instituto Superior Técnico, Lisboa; Faculdade de Farmácia, Universidade de Lisboa; Instituto Nacional de Medicina Legal, Delegação de Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia.

Introduction: Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden death especially among youngsters and athletes, defined by the presence of left ventricular hypertrophy in absence of congenital heart disease or abnormal loading conditions sufficient to cause the observed hypertrophy. It usually shows an autosomal dominant pattern of inheritance, with heterogeneous penetrance, diagnostic and expression, affecting 1:500 individuals. More than 1,000 mutations are associated with this condition, especially in genes encoding sarcomere proteins. Sudden cardiac death (SDC) is one of the most common causes of death and remains a serious public health issue in developed countries. In many cases the cause of death can be established; however the number of SCD, in which no specific cause can be confirmed even thorough a rigorous post-mortem examination, is still highly significant. In the last decade important advances have been made in understanding the genetic basis of these cardiac pathologies. The identification of alterations in the genes can be extremely important in the management of families, especially because it allows clarification of risk situation of asymptomatic family members and, in cases of SCD, it can provide a diagnostic. Recent guidelines recommend the cardiac and genetic screening for family members of patients with HCM or victims of SCD due to cardiac genetic causes. The introduction of new technologies, for mutation detection can provide a more specific and clinically useful genetic testing strategy. New approaches with panels of genes, such as Sequenom MassArray System or Exome Sequencing Technology, are being developed, allowing an increase of the number of genes to be studied simultaneously and detection of a greater variety of mutations. All this information rapidly

increases the rate of variant detection and should enable more families to acquire genotype results. However, this will bring significant challenges for sequence variant interpretation and many variants of uncertain significance are likely to be found.
Methods: During the last 4 years approximately 80 index cases were analysed in the laboratory by direct sequencing or Sequenom MassArray System.
Results: The results of this study demonstrated that the most frequent mutated genes are the *MYBPC3* and *MYH7*. They were detected in more than 50% of the analysed cases, as found in the literature.
Conclusion: New generation technologies promise to revolutionize the current knowledge about genetic bases of HCM and SCD and can contribute to clarify genotype-phenotype co-relation or to establish the cause of death in negative autopsies.

P10. GENETIC CANCER SUSCEPTIBILITY IN DOWN SYNDROME – A PRELIMINARY STUDY

P. Guarino, R. Alves, C. Domingues, J. Leite, M. Ferreira, A.C. Gonçalves, C. Pais, J. Ferrão, I. Carreira, A.B. Sarmento-Ribeiro

Applied Molecular Biology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; Cytogenetic and Genomics Lab, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Down Syndrome (DS) is the most common constitutional aneuploidy and children with DS have an increase 10- to 20-fold risk for leukemia. Folic acid plays a key role in genomic stability and influences gene methylation. The folic acid metabolism may be influenced by dietary habits and genetic polymorphism of Folate Carrier Transporter (RFC), Methyl Tetrahydrofolate Reductase (MTHFR) and cystathionine beta-synthase (CBS) enzymes, playing an important role in susceptibility to aneuploidy and to the development of early events in carcinogenesis.
Objectives: The aims of this study are to analyze the prevalence of polymorphic variants in chromosome 21 genes involved in folate metabolism and the methylation pattern of p16, DAPK, p15 and MGMT genes, in order to identify its role in the development of cancer/leukemia in DS patients.
Methods: For this purpose, we analyzed polymorphic variants CBS 844ins68 and T833C, SOD1 A251G, RFC1 A80G and MTHFR A1298C in 31 children with DS and in 30 healthy controls. Methylation pattern were analyzed in bisulfite converted DNA by MSP.
Results: Our results show a slight decrease in wt 844ins68 CBS allelic frequency in DS (81%), compared to controls (84%). Moreover, we observed that wild type allelic frequency of SOD1, RFC1 and MTHFR polymorphisms (A allele) were similar in controls and DS (SOD1: 92% and 90%; RFC1: 52% and 50%; MTHFR: 70% and 69%, controls and DS). Similar results were observed in SOD1 and MTHFR genotype analysis (SOD1: 83% and 80% AA, 17% and 20% AG and 0% GG; MTHFR: 13% and 10% AA, 33% and 42% AC and 53% and 48% CC, controls and DS). RFC1 AA genotype was decrease in DS (19%) compared to controls (27%). The strength of association between polymorphisms and DS risk was assessed by odds ratio (OR) with the corresponding 95% confidence interval (CI95%). CBS wt 844ins68 (OR = 0.833; CI95% 0.2248-3.089), SOD1 AA (OR = 0.8333; CI95% 0.2248-3.089) and RFC1 AA genotypes (OR = 0,6600; CI95% 0.1980-2.200) could have a protective effect. CBS var. 844ins68 (OR = 1.560; CI95% 0.3927-6.198), SOD1 AG (OR = 1.20; CI95% 0.3237-4.448) and MTHFR AC (OR = 1.444; CI95% 0.5095-4.095) might be a risk factor. Furthermore, p16, DAPK, p15 and MGMT genes were all unmethylated in DS fetus.
Conclusion: Besides, no statistical association was detected between these polymorphisms or the methylation status in children with DS, the increase in patients and controls samples could contribute to a better risk analysis of the influence of these polymorphisms in DS development.

P11. ATYPICAL MICRODELETIONS AND MICRODUPLICATIONS IN 22Q11.2 REGION REVEALED BY ACGH AND MLPA

L.M. Pires, P. Louro, N. Lavoura, A. Estevinho, L. Ramos, I.M. Carreira

Laboratório de Citogenética e Genómica da Faculdade de Medicina da Universidade de Coimbra, Coimbra; Serviço de Genética Médica, Centro Hospitalar e Universitário de Coimbra, Coimbra; Laboratório de Citogenética do Hospital Pediátrico Carmona da Mota, Centro Hospitalar e Universitário de Coimbra, Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra.

Introduction: 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome in humans with an incidence of 1 in 4,000 livebirths. The most frequent clinical features include a dysmorphic facies, conotruncal cardiac defects, hypocalcemia, immune deficiency and palate abnormalities. The reciprocal microduplication on 22q11.2 has been reported, in recent years, as a new genomic syndrome complementary to the 22q11.2DS. The microduplication syndrome phenotype is high variable, ranging from multiple defects to mild learning difficulties, sharing some features with 22q11.2DS, including heart defects, velopharyngeal insufficiency with or without cleft palate, to some individuals being essentially normal. Despite all heterogeneous clinical presentations, the majority of patients with 22q11.2 DS have either a common recurrent 3 Mb deletion or a less common, 1.5 Mb nested deletion, with breakpoint sites in flanking low-copy repeats (LCR) sequences containing at least 30 genes, including the *TBX1* gene. A small number of cases of atypical microdeletions and microduplications with different sizes and locations have also been reported, generally with a milder, slightly different phenotype but with no clear genotype-phenotype correlation to date. The 22q11.2DS diagnostic procedure usually used is Fluorescence *In Situ* Hybridization (FISH) using commercially probes (N25 or TUPLE1), however this test fails to detect those atypical rearrangements that are distal to both FISH probes.
Methods: The implementation in our lab of Array Comparative Genomic Hybridization (aCGH) and Multiplex Ligation dependent Probe Amplification (MLPA) has enabled us to detect less common 22q11.2 rearrangements.
Results: We present 3 families with atypical rearrangements of the distal 22q11.2 region between LCR22-C and LCR22-D, detected by aCGH using SurePrint G3 Human CGH Microarray 4x180K (Agilent Technologies, Santa Clara, CA, USA). Patient 1, a one-year-old boy with cardiac defects, revealed a 731 Kb deletion. Patient 2, a four-year-old boy with psychomotor development delay and facial dysmorphisms, showed a 745 Kb deletion. Patient 3, a male newborn with severe congenital abnormalities including cardiac defects and esophageal atresia, revealed a 588 Kb duplication. These rearrangements were confirmed by MLPA and they were inherited from unaffected parents.
Conclusion: Our results support the phenotype variability and the lack of genotype-phenotype correlation in 22q11.2 chromosomal region. A hypothetical correlation between phenotype, size, associated genes and location of deletions and duplications may be masked by other genetic or epigenetic modifying factors. We suggest that genome-wide microarrays and MLPA will eventually replace FISH as the first technique to detect 22q11.2 rearrangements.

P12. PRENATAL DIAGNOSIS: THE IMPACT OF NEW LABORATORY TECHNOLOGIES

M.C. Pinto, N. Lavoura, A. Mascarenhas, C. Pais, A. Estevinho, P. Paiva, L. Simões, A. Lourenço, A. Jardim, E. Matoso, I.M. Carreira, Equipa de DPN da MBB

Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra; CIMAGO- Centro de Investigação em Meio Ambiente, Genética e Oncobiologia; Centro de Diagnóstico Pré-Natal, Maternidade Bissaya Barreto, Coimbra.

Introduction: Rapid common aneuploidy testing (RAT) (13, 18, 21, X, Y) are widely used in prenatal diagnosis (PD) as a complement of karyotyping. FISH and MLPA can be used for that purpose. Although designed for detection of specific chromosomes, both enable faster results, allowing earlier intervention and reducing parental anxiety.
Objectives: The aim of this work was to evaluate the impact of karyotyping substitution, by RAT, in PD as a stand-alone test, with costs and time benefits.
Methods: The 7035 amniotic fluid (AF) samples received in the laboratory (2006 - 2010), were reviewed. The concordance between results that would have been obtained, by karyotyping and MLPA, if both were applied, successfully, to all the AF samples was determined.
Results: In the study where the concordance between both techniques was determined, all samples considered, showed that the karyotype would have identified 241 chromosome alterations. The MLPA would have detected 117 (48.5%) of these and, in 8.3% of cases, a suspicion of alteration would have existed. The concordance between the two technologies was 98.5%, with 1.5% of false negative results, having 41.3% of the latter a high/ unknown clinical significance. The referrals for the prenatal study, analysed individually, revealed variable values of false negative results from 1% (in advanced maternal age) to 53.1% in cases where one of the parents was a carrier. These results were expected and are in agreement with those previously reported.
Conclusion: The RAT techniques are highly sensitive for the common aneuploidias, are economic and provide faster results. Although, their application in substitution of traditional karyotyping would lead, in some cases, to a wrong diagnosis, with emotional and economic prejudice for not only the affected child's family but also for the State. These techniques are useful and they should be applied as a diagnostic complement.

HEMATO-ONCOLOGY

P13. NON-HODGKIN LYMPHOMA: RARE LOCATIONS, SIMILAR RADIOTHERAPY TREATMENT?

I. Nobre-Góis, J. Casalta-Lopes, A. Silva, M. Jacobetty

Radiation Oncology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra; Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Plasmablastic lymphoma (PL) is an aggressive neoplasm classified as a variant of diffuse large B-cell non-Hodgkin lymphoma (NHL), originally described involving the oral cavity in the clinical setting of HIV and latent EBV infection. PL has been referred, less commonly, in extraoral locations and immunocompetent settings. The prognosis is poor, death typically overcoming in the first two years after diagnosis with current chemotherapy (CHT) and radiotherapy (RT) approaches. Another rare NHL is primary bone lymphoma (PBL) presenting as a lesion, usually confined to a single long bone. Although RT produces local control in over 90% of cases, spread to lymph nodes occurs in approximately 50% of cases. Therefore, combined CHT is usually administered before RT, the prognosis being extremely good. In the very elderly it may be reasonable to treat with RT alone.
Objectives: The aim of this work is to display two clinical cases of NHL with rare locations submitted to RT.
Methods: Two NHL cases with different locations are described. Tridimensional conformal RT planning is illustrated, with tumor and organs at risk delineation. Dose-volume histograms are displayed.
Results: One patient was a 78-year-old male who presented with complaints of nasal obstruction, dizziness and left zygomatic discomfort. The CT scan revealed an expansive lesion occupying the left maxillary sinus, bone-erosive, extending to contiguous tissues, without invading the cranial cavity. The biopsy revealed a high-grade NHL classified as Plasmablastic Lymphoma. The patient underwent RT (administered with a dose of 40 Gy/20 fractions/4 weeks on the lesion with a margin using photons with an energy quality factor of 6 and 18 MV) followed by CHT. The other patient was a 67-year-old female that seeked medical attention for right inguinal pain radiating to the lower limb with shortening and outer rotation on physical examination. The x-ray and CT-scan identified a pathological fracture of the proximal femur. Biopsy revealed a NHL follicular subtype. PET-CT using 2-[^{18}F]-fluoro-2-deoxyglucose (FDG) disclosed an intense uptake in the proximal right femur with bone destruction. The patient underwent "sandwich therapy" with 4 cycles of CHOP (Cyclophosphamide, Hydroxydaunorubicin, Vincristine and Prednisone/Prednisolone), followed by RT (same regimen and radiation energy described above) and 2 more CHOP cycles.
Conclusion: Although having two different clinical conditions, both patients share the singularity of lesion location for each of their NHL subtypes. Notwithstanding their clinical and imaging peculiarities, RT has similar approaches in both cases, and plays an important role in their management.

P14. PET-CT AND RADIOTHERAPY TREATMENT IN NON-HODGKIN LYMPHOMA: A CLINICAL CASE

I. Nobre-Góis, J. Casalta-Lopes, A. Silva, M. Jacobetty

Radiation Oncology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra; Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Positron emission tomography (PET)-Computed tomography (CT) using 2-[^{18}F]-fluoro-2-deoxyglucose (FDG) has been increasingly used in lymphoma management for diagnostic, staging and treatment response evaluation purposes. High-dose radiotherapy (RT) has long been applied in Non-Hodgkin's Lymphoma (NHL) treatment. In 2005 the preferred method for administering RT was "involved field radiation" that targets only lymph node regions that are known to be affected by the disease, sparing the surrounding healthy ones. PET-CT allows more accurate target-volume delineation and lower doses to normal structures, thus reducing morbidity and increasing loco-regional control.
Objectives: The aim of this work is to illustrate the role of PET-CT in a classical clinical case of NHL.
Methods: A NHL clinical case is described. Computerized tridimensional RT planning is illustrated considering tumor location and organs at risk (OAR) in the vicinity of the irradiation field. The role of co-registration of PET-CT to the planning-CT is highlighted. Dosimetric calculi are presented. Treatment outcome and follow-up is mentioned.
Results: The patient was 57 years old and diagnosed with NHL, stage IIIA (Ann Arbor Staging system), in October 2005. The baseline CT-scan showed involvement of bilateral cervical lymph nodes, retro-cava, inter-cavo-aortic, and left inguinal lymph

nodes. He was proposed for chemotherapy (CHT) as first-line treatment with ESHAP regimen (Etoposide, Methylprednisolone/ Solumedrol, high-dose Cytarabine/Ara-C and Cisplatin). After 4 cycles the interim PET-CT revealed a good response to CHT, with active disease limited to a single left cervical lymph node. The patient proceeded with ESHAP and completed the planned number of cycles. The PET-CT performed two months after CHT showed increased uptake of ^{18}F-FDG in the left latero-cervical and supraclavicular lymph-nodes, suggesting disease progression. In multidisciplinary therapeutic decision the patient was proposed for RT. PET-CT was co-registered to planning-CT for more accurate target-volume delineation. A dose-volume histogram was obtained considering OAR. The dose administered was 36 Gy/18 fractions/3.5 weeks on the affected regions using photons with an energy quality factor of 6 and 18 MV. The treatment was well tolerated with a complete response confirmed by PET-CT. The patient has been in follow-up for 6 years and remains in clinical and laboratorial remission.
Conclusion: RT is an important adjunct to combined CHT in NHL. PET-CT allows early response to CHT regimen, better RT planning and end-treatment monitoring. Cooperation between radiation oncologists, nuclear medicine physicians and hematologists is vital to provide optimal treatment with less adverse effects.

P15. EVALUATION OF THE EFFECTS OF X RADIATION IN LYMPHOMA AND LUNG CANCER CELL LINES – THE INTERPLAY BETWEEN OXIDATIVE STRESS AND P53 LEVELS

F. Mendes, R. Silva, A.M. Abrantes, A.C. Gonçalves, M. Laranjo, J. Casalta, P. César, P. Soares, A.B. Sarmento Ribeiro, M.F. Botelho, M.S. Rosa

Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra; Clinical Analysis and Public Department, College of Health Technology of Coimbra; Physics Department, Faculty of Sciences and Technology, University of Coimbra, Coimbra; CIMAGO, Faculty of Medicine, University of Coimbra, Coimbra; CNC, University of Coimbra, Coimbra; Applied Molecular Biology and Clinical University of Hematology, Faculty of Medicine, University of Coimbra, Coimbra; Radiotherapy Service, Hospital and University Center of Coimbra; Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Besides chemotherapy, radiotherapy is commonly used to treat Diffuse Large B Cell Lymphoma (DLBCL) and Small Cell Lung Cancer (SCLC). It's well known that cell irradiation causes direct and indirect effects on cells as a consequence of oxidative stress (OS) generated. This is the result of the disequilibrium between the reactive oxygen species (ROS) produced and the anti-oxidant defenses levels. Many tumors have increased expression of antioxidant molecules, which could enhance the radiotherapy resistance in tumor cells by neutralizing free radicals, being this a major problem associated with radiotherapy failure. Besides that, oxidative stress effects may be mediated by mitochondria dysfunction and/or p53 levels. However theses mechanisms are not clarified in DLBCL and SCLC.
Objectives: To evaluate the effects of X radiation in DLBCL and SCLC cell lines namely in cell viability, proliferation and death. We also wanted to study some of the mechanisms involved, namely the role of OS, mitochondria dysfunction and p53 levels.
Methods: We use the FARAGE (DLBCL) and H69 (SCLC) cells and evaluated cell viability and proliferation in the absence (control) and after exposure to 4 MeV RX with different doses (0.5 Gy, 15 Gy and 30 Gy), every 24 hours for a period of 96 hours, using the tripan blue assay. Cell death and cell cycle were evaluated 48h after Rx exposure by flow cytometry (FC) using the double staining AnV/PI and PI/RNase assay. To evaluate oxidative stress we determined, by FC, the superoxide anion, peroxides and reduced glutathione levels using the probes, dihydroethidium, 2′,7′-dichlorofluorescin diacetate and orange mercury, respectively. We also evaluate mitochondria membrane potential (MMP) by FC, using the fluorescent probe, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazolcarbocyanine, and p53 levels by western blot.
Results and conclusion: Our results suggest that RX induces a decrease in H69 and FARAGE cells proliferation and viability in a dose and time dependent manner, inducing cell death preferentially by apoptosis and/or necrosis. These results are in agreement with the observed cell cycle arrest, increase in ROS levels and decrease in MMP. However it seems that FARAGE cells needs higher radiation doses to obtain the some mitochondrial effects, when compared with H69 cells. These results may relate with the higher basal p53 levels observed in FARAGE cells. Furthermore, we observed an increase in p53 levels in both cell lines submitted to radiotherapy. Our preliminary results suggest that the response of this kind of lymphoma and lung cancer to radiotherapy may by influence by OS and p53 levels.

P16. DO METALLOPROTEINASES PLAY A ROLE IN THE PATHOGENESIS AND PROGRESSION FROM MGUS TO MM?

A.S. Pais, A.C. Gonçalves, C. Geraldes, E. Cortesão, I. Sousa, V. Alves, M. Santos, A. Teixeira, J. Nascimento Costa, A.B. Sarmento-Ribeiro

Medicine Student, Faculty of Medicine, University of Coimbra (FMUC), Coimbra; Applied Molecular Biology Unit, FMUC, Coimbra; University Clinic of Hematology, FMUC, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, FMUC, Coimbra; Immunology Institute, FMUC, Coimbra; Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC/HUC), Coimbra.

Introduction: Multiple myeloma (MM) is characterized by the proliferation of malignant plasma cells (PC) in the bone marrow (BM), increased angiogenesis and development of osteolytic bone disease. The first pathogenic step is a premalignant monoclonal gammopathy of undetermined significance (MGUS), that progress to MM by complex genetic and/or epigentic events occurring in the neoplastic PC and in BM microenvironment. Matrix metalloproteinases (MMPs) are a family of proteinases capable of degrading the extracellular matrix, promoting cell growth, invasion, angiogenesis, metastasis, and bone degradation.
In the present study, we aimed to explore the role of MMPs, namely MMP-2, MMP-8 and MMP-9, in the pathogenesis of MGUS and progression to MM, and correlated these results with clinical/ laboratory data and prognostic factors.
Methods: Expression of MMP-2, MMP-8 and MMP-9 was assessed on BM PC of 17 MGUS and 13 MM newly diagnosed patients and 2 controls by flow cytometry. The average MGUS and MM patients' age is 71 (39-89) and 77 years (68-86), respectively; and 65% of MGUS and 77% of MM patients are female. In MGUS patients the monoclonal protein is 59% IgG, 35% IgA and 6% IgM, while in MM is 55% IgG and 45% IgA. All MM patients have anemia, 47% increased creatinine, 70% bone lesions and 8% have hypercalcemia. According to International Staging System (ISS), 8% of MM patients are in stage I, 23% in stage II and 69% in stage III.
Results: Our preliminary study shows that MGUS and MM patients PC have higher MMP expression levels than controls. But, MM patients show higher percentage of PC expressing MMP-8 and MMP-9 when compared to MGUS. However, the MMP intracellular expression levels are higher in MGUS patients than in MM, especially MMP9. When analyzed both PC patient population in MGUS and MM, we

observed that $CD19^+/CD138^+$ PC have higher MMP intracellular expression levels and percentage of cells expressing MMP, when compared with PC $CD19^-/CD138^+$, especially MMP-2 and MMP-9. All MM patients are positive for at least two MMPs. MMP expression levels are independent of immunoglobulin subtype, but MM patients with osteolytic bone disease have lower MMP intracellular expression than patients without bone disease.
Conclusion: Our findings suggest that PC MMP expression may be correlated with transition of MGUS to MM, promoting extramedullary spreading and disease evolution. The confirmation of these results may contribute to a better understanding of MM biology and can lead to new therapeutic approaches.

P17. EFFECTS OF TRAIL AND SURVIVIN INHIBITORS IN ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT – AN *IN VITRO* SUTY

C. Domingues, J. Leite, M. Ferreira, P. Guarino, R. Alves, M. Coucelo, L. Ribeiro, A.C. Gonçalves, M. Dourado, J.M. Nascimento Costa, A.B. Sarmento-Ribeiro

Applied Molecular Biology and University Clinic of Hematology, FMUC – Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, FMUC – Faculty of Medicine, University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHC/CHUC), Coimbra; Oncology and Paliative Care, FMUC – Faculty of Medicine, University of Coimbra, Coimbra; Internal Medicine Department, Centro Hospitalar e Universitário de Coimbra (HUC/CHUC), Coimbra.

Introduction: T-cell acute lymphoblastic leukemias (T-ALL) are aggressive hematologic tumors resulting from the malignant transformation of T cell progenitors. Despite improved treatment outcome, many patients relapse and this fact may be associated with apoptosis evasion. The cytokine tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL/Apo-2L) can activate apoptosis pathway by binding to its two agonistic cell surface death receptors 4 and/or 5, with low toxicity in normal cells. Nevertheless, in some cases treatment with TRAIL alone may not be sufficient for an effective response. Take into account, combined TRAIL therapies not only with conventional chemotherapeutic agents but also with survivin inhibitors, namely gambogic acid (GA) and silibinin (SLB) have given encouraging results to restore TRAIL sensitivity. In this context, the aim of this study is to evaluate the therapeutic efficacy of a recombinant human TRAIL (rhTRAIL) and survivin inhibitors, GA and SLB, alone and in combination with conventional chemotherapeutic agents, in T-ALL, namely in relapse.
Methods: For this purpose we evaluated the cytotoxic effect of rhTRAIL, GA and SLB in monotherapy, in association with each other and with conventional drugs (Doxorubicin or Vincristine), in two T-ALL cell lines, the CEM and MOLT-4 cells (established from a patient at disease presentation and relapse, respectively). Cell viability was assessed by trypan blue assay and cell death by Optical Microscopy and flow cytometry (FC) using the Annexin V/Propidium Iodide staining. TRAIL, TRAIL-Receptors, survivin, Transferin receptor (TfR), activated caspase-3 and cytocrome *c* expression levels were also evaluated by FC using monoclonal antibodies. Mitochondrial potential was assessed by FC using JC-1 probe.
Results: Our results show that, as single agent, rhTRAIL, GA and SLB induce antiproliferative and cytotoxic effects in a dose, time, administration and cell type dependent manner. Furthermore, the therapeutic association induces synergist cytotoxic effect in both cell lines. The different therapeutic efficacy of rhTRAIL, GA and SLB, in both cell lines may also be correlated with the ratio between TRAIL pro- and anti-apoptotic receptors, TfR and survivin basal expression levels, respectively, which are higher in CEM than in MOLT-4. These compounds induce cell death mainly by apoptosis with mechanisms may be related with mitochondrial apoptotic pathway activation.
Conclusion: Our study suggests that rhTRAIL and survivin inhibitors, in monotherapy or in combination, may constitute a new potential therapeutic approach to overcome treatment failure and relapse in T-ALL treatment.
This work was supported by CIMAGO (Project 02/08).

P18. HYDROGEN PEROXIDE AND MENADIONE ALTER DNA METHYLATION STATUS IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS *IN VITRO*

J. Leite, M. Ferreira, P. Guarino, C. Domingues, R. Alves, A.C. Gonçalves, A.B. Sarmento-Ribeiro

Applied Molecular Biology and University Clinic of Hematology, FMUC – Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, FMUC, Coimbra FMUC; Centro de Neurociências e Biologia Celular (CNC), Universidade de Coimbra, Coimbra.

Introduction: Acute lymphoblastic leukemia (ALL) originates from the malignant transformation of lymphocyte progenitor cells into leukemic cells in the B-cell and T-cell lineages. Increasingly evidence shows that oxidative stress and reactive oxygen species (ROS) are involved in carcinogenesis, since they can cause DNA damage. Moreover, it is now clear that epigenetic mechanisms are as important as genetic changes in the development of cancer. Same studies suggest that oxidative DNA damage can affect patterns of DNA methylation leading to aberrant gene expression and possibly contributing to the development of malignancy. In this work, we study the effect of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) in ALL, with special emphasis on the action of oxidative stress (OS) in cell death and methylation status.
Methods: CEM cells (T-ALL cell line) were treated in the absence and presence of hydrogen peroxide and menadione (O_2^- donor) and cell viability and density were analyzed by trypan blue assay. Intracellular levels of H_2O_2, O_2^-, GSH and mitochondrial potential were determined by flow cytometry (FC), using the fluorescent probes. Cell death and were also evaluated by optical microscopy and FC using the Annexin V/Propidium Iodide staining. We also analyzed apoptotic proteins expression levels, namely BAX, BCL-2, FAS, FAS ligand and caspases, and cell cycle by FC. Global DNA methylation and hydroxymethylation were analyzed by ELISA using commercial kits.
Results: Our results show that H_2O_2 and menadione decrease cell viability in a dose and time dependent manner. In fact, we observe that IC_{50} of H_2O_2 and menadione in CEM cells is 25 μM and 7.5 μM, respectively, after 24 hours. These compounds induce cell death mainly by late apoptosis/necrosis, with decrease in mitochondrial membrane potential and increase in caspases levels. BAX/BCL-2 and FAS/FAS ligand ratios were increased in cells treated with H_2O_2 and menadione, respectively. OS may mediate these effects since we observe an increase in ROS levels and a decrease in GSH. These compounds induce S phase arrest and an increase in 5-hydroxymethylcytosine (5hmC) levels in cell treated with 25 μM H_2O_2. This fact can be result of 5-methylcytosine oxidation via oxidative damage, and consequently induction of global hipomethylation. In contrary, in cells treated with 7.5 μM de menadione 5hmC levels decrease.
Conclusion: In conclusion, our results suggest that, despite the influence of ROS in cell death, oxidative stress levels may lead to changes in global DNA methylation status.

P19. SCREENING OF ABL KINASE DOMAIN MUTATIONS USING HIGH-RESOLUTION MELTING IN CHRONIC MYELOID LEUKAEMIA: IMPACT IN THE RESPONSE TO TREATMENT WITH TYROSINE KINASE INHIBITORS

P. Couceiro, M. Ferreira Teixeira, P. Freitas Tavares, P. Rodrigues Santos

Immunology and Oncology Lab, Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra; Hematology Service, Centro Hospitalar Universitário de Coimbra, Coimbra; Immunology Institute, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: BCR-ABL fusion protein is the central therapeutic target in chronic myeloid leukemia (CML) using tyrosine kinase inhibitors (TKIs), a paradigm for molecular therapy that have improved prognosis in CML. Mutations in ABL kinase domain (KD) are associated with the resistance to TKIs in CML patients. Sanger sequencing is the gold standard method for KD mutation detection. However, High-Resolution Melting (HRM) PCR techniques present a significantly better sensitivity (1%) when compared to conventional Sanger sequencing (c.a. 20%).
Objectives: This study aimed to test HRM as a rapid screening prior to ABL KD sequencing in CML patients.
Methods: The present study included 62 different CML patients followed quarterly at the Hematology Service of CHUC. A total of 494 peripheral blood samples collected in Paxgene RNA blood tubes were analysed by 4 distint overlapping HRM PCR reactions, using specific primers covering the entire ABL KD region.
Results: From routine nested-PCR and RT-qPCR BCR-ABL analysis, 15.2% (75/484) samples were found positive for p210 BCR-ABL transcripts. In 13.3% of the samples (10/75) mutations were suggested from HRM profiles. Sanger sequencing confirmed T315I mutations in 20% of these samples (2/10).
Conclusion: Sequencing is an expensive and a time-consuming procedure to test for ABL KD mutations. Therefore, routine HRM PCR strategy is a sensitive, simple, fast and cost effective method that should be considered for initial quick screening of ABL KD mutations in CML patients.

P20. IL-2, IL-12 AND IL-15 PREVENT REACTIVE OXYGEN SPECIES ACCUMULATION ON NATURAL KILLER CELLS FROM CHRONIC MYELOID LEUKEMIA PATIENTS FOLLOWING INTERFERON-ALPHA, IMATINIB AND DASATINIB THERAPY

D. Silva, P. Couceiro, M. Ferreira-Teixeira, P. Tavares, H. Sá, P. Rodrigues-Santos

Instituto de Imunologia, Faculdade de Medicina, Universidade de Coimbra, Coimbra; Lab Imunologia e Oncologia, Centro de Neurociências e Biologia Celular, Universidade de Coimbra; Hematologia Oncológica, Centro Hospitalar Universitário de Coimbra, Coimbra.

Introduction: Extracellular reactive oxygen species (ROS) produced by phagocytes are the main responsible for the immunosuppressive state of NK cells in chronic myeloid leukemia (CML), due to the uncontrolled expansion of myeloid cells. NK cells from CML patients have decrease expression of membrane receptors necessary for the cytotoxic action over BCR-ABL positive cells.
Objectives: We aimed to investigate alterations of ROS levels in NK cells of CML patients due to oxidative burst from phagocytes, taking into account the type of treatment and disease progression.
Methods: In this work, we analyzed 90 peripheral blood samples from 50 CML patients. CML patients were under IFN-α, Imatinib or Dasatinib therapy. Production of ROS was evaluated using flow cytometry by the conversion of dihydrorhodamine 123. In addition, peripheral blood NK cells and monocytes from 20 healthy individuals were sorted and cocultured under different conditions, to evaluate NK cell capacity to resist to ROS production.
Results: CML patients have higher percentage of NK cells in the lymphocyte population (17.04 ± 9.3) compared with healthy controls (11.9 ± 5.4). However, the expression of NKp46 and CD16, two important receptors in antitumor response, was significantly decreased. NK cells had chronic increased ROS levels in blood of CML patients, similar to those levels of healthy controls after *in vitro* PMA stimulation. No significant difference was found comparing $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ NK cell subsets or patients undergoing major cytogenetic response vs. molecular complete response. Tyrosine kinase inhibitors were found to influence ROS levels in NK cells, mainly in patients treated with Imatinib 600 mg. Interferon-alpha revealed a minor impact on ROS. Interestingly, stimulation of NK cells with IL-2, IL12 and IL-15 before contact with stimulated monocytes revealed a protective role, decreasing ROS accumulation in NK cells.
Conclusion: NK cells from CML patients present a significant increase of ROS levels. A combination of costimulatory cytokines (IL-2, IL-12 and IL-15) prevents NK cells from immunosuppressive state induced by myeloid cells. Refinement of current therapeutic protocols increasing NK cell functional properties seems to be a promising field to explore in CML therapy.

P21. TYROSINE KINASE FLT3 AND C-KIT RECEPTOR EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

E. Cortesão, A.C. Gonçalves, I. Sousa, C. Moucho, J. Carda, C. Geraldes, N. Costa e Silva, M.A. Pereira, A. Teixeira, G. Ribeiro, J. Nascimento Costa, A.B. Sarmento Ribeiro

Serviço de Hematologia, Hospitais da Universidade de Coimbra, Coimbra; Faculdade de Medicina da Universidade de Coimbra, Coimbra; Serviço de Patologia Clínica, Hospitais da Universidade de Coimbra, Coimbra; Serviço de Medicina, Hospital Distrital da Figueira da Foz, Figueira da Foz; Centro de Investigação em Meio Ambiente, Genética e Oncobiologia.

Introduction: Myelodysplastic Syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by dyshematopoiesis and high risk of developing acute myeloid leukemia. Genetic and/or epigenetic mechanisms can contribute to the pathogenesis of gene dysfunction in MDS. Over the last decade, major signal transduction pathways triggered by exogenous stimuli were identified and characterized, with the FLT3 and c-KIT signaling pathways playing a key role. FLT3 and c-KIT are important members of the receptor tyrosine kinase family that are overexpressed in many malignant hematologic diseases. However, little is known about the role of these proteins in MDS.
Objectives: Investigate the role of the expression of tyrosine kynase receptors, FLT3 and c-KIT, and gene mutations, in MDS patients and their correlation with clinical prognosis.
Methods: We analyzed 12 MDS *de novo* patients, the median age was 72 years (22-89), gender M/F = 5/7, WHO subtypes: RCMD (n = 6), RA (n = 3), RARS (n = 1), RAEB-2 (n = 1), CMML (n = 1) and IPSS: low (n = 6), intermediate-1 (n = 5) and intermediate-2 (n = 1). We examined c-KIT protein expression by flow cytometry using a monoclonal anti-CD117 antibody, in CD34 bone marrow cells collected from MDS patients at diagnosis and in 5 non-neoplastic patients (controls). The *c-KIT* D816 and the *FLT3* gene mutations, Internal Tandem Duplications (ITD) and the D835 mutation, were analyzed by PCR-RFLP.
Results: Our preliminary results show an increase in c-KIT expression in CD34 positive cells in MDS patients as compared with controls. The percentage of c-KIT protein expressing cells

was also higher, in particular in CD34 negative cells. On the other hand, c-KIT protein expression seems to be correlated with the WHO MDS classification and IPSS, being highest in RCMD and INT-1 MDS prognostic group respectively. Furthermore, we didn't find any *FLT3* mutations in our population, but in the RAEB-2 patient we observed the *c-KIT* D816 mutation, and this patient progressed to acute leukemia.
Conclusion: These preliminary results suggested that the elevated c-KIT expression could maintain the MDS clone and could be helpful to the pathogenesis and prognosis prediction of MDS patients. However further data and refinement of analysis are needed to confirm our results ant to predict clinical outcomes.
This work was supported by CIMAGO (Project 24/09).

P22. WNT/B-CATENIN, NOTCH AND HEDGEHOG PATHWAYS – POSSIBLE NEW TARGETS TO ALL THERAPY

M. Ferreira, P. Guarino, C. Domingues, J. Leite, R. Alves, A.C. Gonçalves, A.B. Sarmento-Ribeiro

Applied Molecular Biology and University Clinic of Hematology, Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, Faculty of Medicine, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra.

Introduction: Conserved embryonic signaling pathways such as Hedgehog (Hh), Wingless (Wnt) and Notch, critical for stem cell self-renewal and differentiation in hematopoiesis, have been implicated in the pathogenesis of several hematological malignancies. Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can develop from the aberrant activation of the Wnt/β-catenin, Notch and Hedgehog signaling pathways. On account of that, these pathways may constitute new potential candidate targets for ALL therapy.
Objectives: The main goal of this study was to evaluate the therapeutic potential of Wnt/B-catenin, Notch and Hedgehog inhibitors, respectively IWR-1, gamma-secretase inhibitor XXII (GSI) and GDC-0449, alone and in combination in an ALL cell line.
Methods: To evaluate the effect of these developmental signaling pathways inhibitors on cell viability, we use an ALL cell line, the CEM cells, submitted to different concentrations of the inhibitors. The IC50 (half maximal inhibitory concentration), was determining using the blue trypan assay. The cell death was assessed by optical microscopy (May-Grunwald staining) and by flow cytometry (Propidium Iodide/Annexin V staining, BAX and BCL-2 levels and mitochondrial membrane potential). We also analysed, by flow citometry, some proteins related with cell cycle regulation, as p53 and Cyclin D1.
Results: The results observed showed that, in CEM cells, GSI, IWR-1 and GDC-0449 induced cytostatic and cytotoxic effects. These inhibitors suppressed cell growth and induced a decrease in cell viability in a time- and dose-dependent manner, when administrated alone or in combination with each other. The half maximal inhibitory concentration (IC_{50}) of GSI IWR-1 and GDC-0449 was 50 μM, 30 μM and 150 μM, respectively, after 24h of treatment. These compounds induce cell death mainly by apoptosis, that may related with observed increase in caspses levels and decrease in mitochondrial membrane potential and BAX/BCL-2 levels. We also observe that in the presence of these pathways inhibitors, p53/cyclin D1 levels where diminished and that they have not a notable influence in cell cycle arrest.
Conclusion: In conclusion, our results suggest that GS, IWR-1 and GDC 0449 are potentially new targeted therapies that could be efficient in ALL treatment.

P23. EXTRANODAL NK/T CELL LYMPHOMA, NASAL TYPE: CASE REPORT AND REVIEW OF LITERATURE

M. Silva, M. Santos, S. Augusto, A. Cunha, S. Paiva, J. Migueis, J.C. Ribeiro, J. Romao, A. Paiva

Otorhinolaryngology Department; Hematology Department; Hospitais da Universidade de Coimbra, Coimbra.

Introduction: Nasal type natural killer cell lymphoma is characterized by progressive destruction of midfacial structures (previously known as lethal midline granuloma). It is an extremely rare malignancy in the west (< 1% of all lymphomas) but much more frequent in Asian and Latin American countries (where it constitutes 7 to 10%). Little is known about the etiology, although there is a strong association with Epstein Barr Virus, suggesting a probable pathogenic role of the virus. Bone marrow involvement *ab initio* is quite rare (< 10%).
Methods: Case report of a male patient diagnosed NK/T cell lymphoma with bone marrow involvement at diagnosis in our department. A critical appraisal of current literature was performed.
Results: A Caucasian 65 years old patient presented to the ENT emergency room with an extensive nasal mass, ulcerative palatal midline destruction and cervical lymphadenopathy. Biopsy confirmed a nasal type NK/T cell lymphoma with necrosis and an angiocentric growth pattern. CT scan and PET scan revealed bilateral cervical nodes and a bone marrow involvement at left femur, right clavicle and 9th dorsal spine vertebra. Detection of Epstein-Barr virus DNA in peripheral blood was positive. Patient received a first cycle of chemotherapy and went into remission. A few days later he developed a progressive disease with no response to additional medical management and deceased.
Conclusion: An ulcerative lesion in the midline head and neck region must have extranodal NK/T-cell lymphoma as a differential diagnosis even though it is extremely rare in our country. Several biopsies may be required to confirm the diagnosis due to extensive inflammatory infiltrates, tissue necrosis and poor quantity of atypical lymphocytes. Currently, treatment is based on chemotherapy and radiotherapy but prognosis remains poor.

SOFT TISSUE AND BONE CANCER

P24. GIANT CELL TUMOUR OF BONE - 14 YEARS OF EXPERIENCE ON IT'S TREATMENT

J.P. Oliveira, R. Fonseca, A. Laranjo, F. Fonseca, J. Casanova

Serviço de Ortopedia, Centro Hospitalar e Universitário de Coimbra, Coimbra.

Introduction: Giant Cell Tumour of Bone (GCTB) is a relatively common tumour. Benign in nature, but with local aggressiveness, these bone tumours are characterized by their tendency for bone destruction and high rate of local recurrence. Detection and treatment of these tumours assumes particular importance as it will influence the final outcome, with direct repercussions on the clinical and functional prognosis of the patient. The aim of this study is to make a critical analysis of the cases operated in our department on the last 14 years.
Methods: We conducted a retrospective and case series study of the last 79 GCTB operated on the various stages of disease between 1996-2010. With a minimum postoperative follow-up of 2 years, the primary endpoint was to critical analyse the surgical option in terms of the rate of complications and recurrence. Campanacci grading

system was used for surgical staging and a statistic study was made. A p-value < 0.05 was considered statistically significative.
Results: The median age at diagnosis was 39 ± 16.5 years [15-79] and the majority (62%, n = 49) of the population were females. About location, the proximal tibia has the greatest number of cases (34.2%, n = 27). The most frequent symptom was pain (62.0%, n = 49) and in 9 cases (11.4%) the first symptom was a pathological fracture. The average time between diagnosis and surgery was 54.5 ± 136.2 days [2-242]. According to Campanacci classification, 36.7% (n = 29) were classified as belonging to Grade III and 24 1% of the cases were not classified. The most frequent type of resection used was the intramarginal (53.2%, n = 42) and for reconstruction the preference was on using autograft associated with polymethylmethacrylate (37.7%, n = 23). In 2 cases Denosumab was used with good outcomes achieved. About complications, on the majority of the patients (86.1%, n = 68) were characterized as low or absent. With a postoperative follow-up of 7,8 ± 4,9 years [2-16], there were 17.7% (n = 14) cases of recurrence but from these only 35.7% (n = 5) were primarily treated in our department. We were unable to establish a statistically significant relationship between Campanacci classification nor time between diagnosis and surgery versus recurrence rate. There was no case of multicentre or metastatic disease.
Conclusion: The work in question revealed a low recurrence rate on the treatment of GCTB and no cases of multicentre or metastatic disease, which in our opinion is due to the short time between diagnosis and surgery, the choose of the best case-on-case surgical option and the therapeutic expertise of the surgical department.

P25. THORACIC MALIGNANT SOLITARY FIBROUS TUMOR: WHEN SURGERY WAS NOT AN OPTION

J. Casalta-Lopes, I. Nobre-Góis, T. Teixeira, M. Borrego, P. Soares

Radiation Oncology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra; Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Solitary fibrous tumor of the pleura (SFTP) is a rare neoplasm, with an incidence of approximately 2.8 cases out of 100,000 hospitalized patients. It occurs in all ages, with a peak between 30 and 60 years. Approximately 10% to 20% of all cases reported in literature are malignant (mSFTP), and larger tumors are more likely so. Primary treatment of patients with mSFTP is complete surgical resection. As recurrence rate is high, aggressive surgery is recommended and careful follow-up (FU) is mandatory. Up to date, no definitive criteria have been defined regarding adjuvant treatment; in this setting, further investigations are strongly needed. Radiotherapy (RT) appears to be indicated only when surgical resection is not possible or incomplete; however, data is insufficient to define its role in the disease management.
Objectives: The aim of this work is to describe a clinical case of unresectable mSFTP and the use of RT.
Methods: A case of mSFTP is exposed. Conformal tridimensional RT planning is depicted, with tumor and organs at risk (OAR) delineation. Treatment outcome and FU are presented.
Results: A female patient with 65 years presented to her assistant cardiologist with flu-like symptoms that had been present for three months. Chest X-ray showed an opacity occupying most of the right hemi-thorax. The subsequent computed tomography (CT) scan revealed a solid tumor mass with 16cm in greater dimension, without separation planes with the mediastinum, superior vena cava, trachea and esophagus, and therefore unresectable. The biopsy revealed a mSFTP. Given the priors of coronary artery disease the patient was not an adequate candidate to perform chemotherapy. Consequently, she was proposed for RT in multidisciplinary therapeutic decision. A dose of 45 Gy/25 fractions/5 weeks was administered on the lesion with a margin using photons with an energy quality factor of 18 MV. On CT scan performed three weeks after RT completion, the mass had suitable dimensions and cleavage planes for surgical approach to be attempted. A right superior lobectomy was performed with complete resection of the mass. Pathology confirmed the diagnosis. Five months after surgery the patient was disease-free with evidence of radiation pneumonitis on CT-scan, clinically unapparent.
Conclusion: Although RT may not be established as first-line treatment in mSFTP, this case shows its usefulness in unresectable tumors, as it allowed surgery to be performed.

P26. THE USE AND ACCURACY OF CT AND ULTRASOUND-GUIDED BIOPSIES IN MUSCULOSKELETAL PATHOLOGY

R. Fonseca, N. Magalhães, A. Agostinho, F. Alves. H. Garcia, J. Freitas, A. Laranjo, J. Casanova

UTAL-HUC, Imagiologia-HUC, Anatomia Patológica-HUC.

Introduction: CT and Ultrasound-guided biopsies of musculoskeletal tumors are easily performed, under local anesthesia, with minor discomfort to the patient. This proceeding plays an important role in the diagnosis and treatment planning of musculoskeletal tumors.
Objectives: The main goal of our study was to evaluate the diagnostic accuracy of CT and Ultrasound guided biopsies in musculoskeletal pathology and their relation with final histology and anatomic location of the lesion.
Methods: Between January 1998 and December 2010, we have performed 1,177 biopsies through intervention radiology. To evaluate the accuracy of this procedure the diagnosis obtained in the biopsy was compared with the respective surgical specimens. The number of biopsies needed to obtain a diagnosis was also included in the study.
Results: Intervention radiology biopsies have a global accuracy of 85% (997/1,177). Diagnoses that were uncorrected were 4% (44/1,177) and inconclusive results were found in 12% (136/1,177) of cases. The global accuracy rates were 86% for benign lesions, 83% for malignant lesions and 96% for secondary lesions. A greater diagnostic accuracy was found in soft tissue lesions in the lower limb (p = 0.018). Also in the soft tissue lesions a lower accuracy was related with the scapular location (p = 0.037). A better diagnostic accuracy was found in the soft tissues tumors when compared with bone lesions.
Conclusion: CT and ultrasound biopsies are a safe method with a high accuracy rate for the diagnosis of bone and soft tissue lesions. The diagnostic global accuracy of 85% obtained in our study demonstrates the validity of this diagnostic method in musculoskeletal pathology.

CNS CANCER

P27. NF2 TUMOR SUPPRESSOR GENE MUTATIONS IN SPORADIC MENINGIOMAS

P.H. Domingues, Á. Otero, M. Jara-Acevedo, P. Sousa, A.B. Nieto, A. Rodríguez Caballero, C. Lopes, A. Orfao, M.D. Tabernero

Centre for Cancer Research (CIC IBMCC-CSIC/USAL) Salamanca; Research Laboratory of the University Hospital of Salamanca, IBSAL; Neurosurgery Service of the University Hospital of Salamanca, Salamanca; Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra; Faculty of Pharmacy, University of Coimbra, Coimbra; IECSCYL, Spain.

Introduction: Meningiomas are central nervous system tumors which are known to contain a recurrent genetic alteration - monosomy 22/

del(22q) -, with the NF2 gene being described as the most involved in the development of the tumor. Although monosomy 22/del(22q) is present in around half of all meningiomas, and NF2 mutations are also frequently found in these tumors, no study has been reported so far in which both alterations are simultaneously assessed and correlated with the other features of the disease.
Methods: In the present study we analyzed the frequency of both copy number changes involving chromosome 22 and NF2 mutations in 20 sporadic meningiomas using single nucleotide polymorphism (SNP)-arrays, interphase-fluorescence *in situ* hybridization (iFISH) and PCR techniques.
Results: Our results show a significant frequency of NF2 mutations (6/20 patients, 30%), most of which (5/6) had not been previously reported in sporadic meningiomas. NF2 mutations involved five different exons – c.469_487dup, c.186delT, c.841delG, c.855delT, c.1165delC and c.357_359del in the exon 5, 2, 9, 12 and 3, respectively - and they all leaded to a truncated protein (p.Leu163Cys, p.Phe62Leu, p.Asp281Met, p.Phe285Leu, p.Gln389Arg and p.Phe119del). Additionally, iFISH showed losses on chromosome 22 in 55% of the cases (11/20), consisting either on monosomy 22 (9/11 cases) or del(22q) (2/11 cases). Interestingly, all NF2 mutated cases were female patients with a higher median age and associated with monosomy 22 but not del(22q).
Conclusion: These results confirm and extend on previous observations about the high frequency and heterogeneity of NF2 mutations in sporadic meningiomas and indicate they could be restricted to a well-defined cytogenetic and clinical subgroup of menopausal women. Further studies in large series of patients are required to confirm our observations.
Grants: FCT, Portugal: SFRH/BD/64799/2009; Consejería Sanidad Junta de Castilla y León, Gerencia Regional de Salud: GRS689/A/11; Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Madrid, Spain: RTICC RD12/0036/0048.

P28. CHROMOSOMAL ABNORMALITIES IN GLIOBLASTOMA MULTIFORME AND THEIR IMPACT ON GENE EXPRESSION

I. Crespo, H. Tão, A.B. Nieto, O. Rebelo, A.L. Vital, M.C. Patino, M. Barbosa, M.C. Lopes, A. Orfao, M.D. Tabernero

Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra; Faculty of Pharmacy, University of Coimbra, Coimbra; Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra; Neurosurgery Service, University Hospital of Coimbra, Coimbra; Center for Cancer Research (CIC IBMCC-CSIC/USAL) Salamanca; Neuropathology Laboratory, Neurology Service, University Hospital of Coimbra, Coimbra; Department of Statistics, University of Salamanca; Faculty of Medicine, University of Coimbra, Coimbra; Department of Medicine, University of Salamanca; Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL); IBSAL-Research Laboratory of the University Hospital of Salamanca.

Introduction: Glioblastoma multiforme (GBM) is the most common malignant subtype of glial tumors, displaying a variable and unfavorable prognosis. Tumor development involves functional inactivation of tumor suppressor genes and DNA repair genes, and/or activation of oncogenes. However, the pathogenesis of GBM is still far from being completely understood, as the relevance of novel candidate genes remains to be elucidated.
Objectives: The aim of this study was to analyze the frequency of recurrent amplicons/homozygous deletions in GBM, and to determine the impact of copy number (CN) alterations on the expression levels of the genes contained therein, as well as on disease outcome.
Methods: We performed whole-genome DNA analysis using high-density single nucleotide polymorphism (SNP)-arrays (Affymetrix 500K and SNP 6.0) in 57 GBM samples, and massive mRNA analysis through cDNA-microarrays (Affymetrix U133Plus2.0), in a subset of 23 tumors. Additionally, confirmatory interphase fluorescence *in situ* hybridization (iFISH) and RT-PCR studies were performed to validate the results. Tumor samples were screened for specific DNA CN alterations involving chromosomal regions of gain and loss: gains of chromosome 7 (97%), and losses of chromosomes 9p (83%) and 10 (91%) were the most frequent alterations. Once the alterations detected for these three chromosomes were simultaneously considered, five distinct cytogenetic profiles emerged, which identified distinct prognostic subgroups of glioblastomas. Of note, those tumors displaying *EGFR* amplification clearly showed a better outcome among older (> 60 years old) patients, as confirmed by further multivariate analysis of a larger series (n = 119) of primary GBM from public databases.
Results: Whole genome analysis also revealed recurrent amplicons for chromosomes 7 (50%), 12 (22%), 1 (11%), 4 (9%), 11 (4%), and 17 (4%), while recurrent homozygous deletions involved chromosomes 9p21 (52%) and 10q (22%). Interestingly, most genes which displayed a high correlation between DNA CN values and mRNA levels were coded in amplified regions, pointing out the potential role of several genes in chromosomes 12q14 (e.g. *RAP1B*, *MDM2* and *GRIP1*) and 4q12 (e.g. *TMEM165*, *FIP1L1* and *EXOC1*), in addition to the *EGFR* gene (7p11.2). Conversely, for the homozygously deleted regions, such correlation was restricted to the *MTAP* gene (9p21).
Conclusion: In summary, the combination of high-throughput genomic and transcriptional data is crucial to define recurrent cytogenetic profiles in GBM, and to disclose those genes showing concordant CN alterations and expression patterns, that may have functional relevance in the pathogenesis of glioblastoma. Further studies in larger series of patients are required to confirm the potential clinical significance of our findings. To confirm our observations.
Grants: FCT, Portugal: SFRH/BD/64799/2009; Consejería Sanidad Junta de Castilla y León, Gerencia Regional de Salud: GRS689/A/11; Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Madrid, Spain: RTICC RD12/0036/0048.

DIGESTIVE CANCER

P29. TARGETING SIGNALING PATHWAYS AS A NEW THERAPEUTIC APPROACH IN HEPATOCELLULAR CARCINOMA CELL LINES

M. Martins, S. Neves, A.B. Sarmento-Ribeiro, J.M. Nascimento Costa

Faculty of Medicine, University of Coimbra (FMUC), Coimbra; Center of Investigation on Environment, Genetics and Oncobiology (CIMAGO), FMUC, Faculty of Medicine, University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; University Hospital of Coimbra (HUC/CHUC), Coimbra.

Introduction: Hepatocellular carcinoma (HCC) is a highly prevalent and lethal neoplasia. Despite its impact, there are only limited therapeutic options, many with negligible clinical benefit, since it is often diagnosed at advanced stages. Besides that, it is highly resistant to currently available chemotherapeutic agents, being also rarely amenable to radiotherapy. In fact, advances in the understanding of tumor biology open new paths for the prevention and treatment of HCC through the development of

targeted therapies. The design of drugs that regulate cancer-related pathways, such as inhibitors of proliferation or activators of apoptosis are essential to the opening of new horizons in the HCC treatment.
Objectives: The aim of this study was to evaluate the therapeutic potential of mTOR (Everolimus), farnesiltransferase (L-744,832) and proteasome inhibitors (MG-262) as new targeted therapies in HCC cell lines in monotherapy and in combination with conventional chemotherapy.
Methods: For this purpose, two HCC cell lines with different p53 status, the HepG2 and HUH-7 cells, were cultured in absence or presence of increasing concentration of Everolimus, L-744,832 and MG-262. The cytotoxic effect was assessed by the Alamar Blue assay and the mechanisms of cell death by optic microscopy (after May-Grünwald-Giemsa staining) and flow cytometry (Annexin V/ Propidium Iodide assay). The molecular mechanisms involved in drug cytotoxicity, namely the expression of ubiquitin conjugates, laminin A/C, cyclin D1 and proteins related to cell death (BAX and BCL2), were analysed by flow cytometry using monoclonal antibodies labeled with fluorescent probes. We also evaluated the implication of these drugs in cell cycle progression, by flow cytometry (IP/RNase).
Results: Our results showed that mTOR, farnesiltransferase and protesome inhibitors had antiproliferative and cytotoxic effects in monotherapy in a dose, time and cell line dependent manner, inducing cell death preferentially by apoptosis. Furthermore, combination of Everolimus, L-744,832 and MG-262 in lower doses than the IC50 with conventional chemotherapeutic drugs demonstrated a synergistic cytotoxic effect allowing to reduce the toxicity levels and side effects, which are critical to improve patients survival and quality of life.
Conclusion: Our study suggests that mTOR, farnesiltransferase and proteasome inhibitors, may constitute a new therapeutic approach in HCC, independently of p53 status, either in monotherapy or in association with conventional chemotherapy.

P30. NEW PERSPECTIVES ON HEPATOCELLULAR CARCINOMA TREATMENT: THE ROLE OF GOSSYPOL

A.F. Brito, A.M. Abrantes, M. Ribeiro, A.C. Gonçalves, A.B. Sarmento-Ribeiro, F. Castro-Sousa, J.G. Tralhão, M.F. Botelho

Biophysics Unit, IBILI - Faculty of Medicine, University of Coimbra, Coimbra; Center of Investigation on Environmental, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra; Faculty of Sciences and Technology, University of Coimbra, Coimbra; Applied Molecular Biology and Hematology Group, Faculty of Medicine, University of Coimbra, Coimbra; Surgical Department, Surgery A, HUC, Coimbra.

Introduction: Gossypol, a natural compound extracted from the cotton plant has been shown to inhibit the growth of several tumour cell lines, including Hepatocellular Carcinoma (HCC) cells. This compound is a potent inhibitor of Bcl-2 family of antiapoptotic proteins. On the other hand it is known that gossypol is a competitive inhibitor of GLUT1 whose expression is increased in HCC and promotes tumorigenesis.
Objectives: This study aims to test the anticancer effect of gossypol in three HCC cell lines, study its effect on Bax and Bcl2 expression as well as check its effect on 18F-FDG uptake (an glucose analogue). We also intend evaluated the effect of gossypol on cell cycle.
Methods: The cell lines used are HepG2 (wp53), HuH7 (mp53) and Hep3b2.1-7 (p53 null). Cell lines were incubated with gossypol in several concentrations. Cell proliferation was evaluated by MTT test. The type of cell death and the percentage of live cells were assessed by flow cytometry. Bax, Bcl2 and GLUT1 expression and cell cycle was also assessed by flow cytometry. For uptake studies, 18F-FDG was incubated in a cell suspension in cells pre-incubated with gossypol and control cells. Samples were collected to eppendorf tubes for tracer uptake calculation. Eppendorfs were then centrifuged and radioactivity of cell pellets and supernatants was measured with a well-type gamma counter.
Results: The concentration necessary to achieve the IC50 is higher for HuH7 cells. More sensitive cell line is Hep3B2.1-7. Flow cytometry results show that gossypol induces high apoptosis in HepG2 and HuH7 cells. In Hep3B2.1-7 cell line there is a balance between apoptosis and necrosis. This compound also induced Bax activation in all cell lines. Gossypol causes a delay in pre-G1 phase on cell cycle. For the three cell lines studied, gossypol was able to decrease the percentage of 18F-FDG uptake.
Conclusion: Gossypol has anti-proliferative effect on HCC. The decrease of cell proliferation could be associated with lower glucose uptake, as was shown with 18F-FDG uptake. Cell death occurs primarily by apoptosis through Bax activation. This compound could help to overcome resistance to chemotherapy and radiotherapy in this type of tumor, contributing to the existence of a personalized therapy.

P31. QUERCETIN: AN OPTION IN HEPATOCELLULAR CARCINOMA THERAPY?

A.F. Brito, M. Ribeiro, A.M. Abrantes, A.C. Gonçalves, A.B. Sarmento-Ribeiro, F. Castro-Sousa, J.G. Tralhão, M.F. Botelho

Biophysics Unit, IBILI - Faculty of Medicine, University of Coimbra, Coimbra; Center of Investigation on Environmental, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra; Faculty of Sciences and Technology, University of Coimbra, Coimbra; Applied Molecular Biology and Hematology Group, Faculty of Medicine, University of Coimbra, Coimbra; Surgical Department, Surgery A, HUC, Coimbra.

Introduction: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. Glucose transporter-1 (GLUT1) expression is increased in HCC and promotes tumorigenesis. Flavonoids, including quercetin, have shown potential as GLUT1 function inhibition and they can be useful as therapeutic weapons against this highly aggressive kind of tumor. The aim of this study is to evaluate the potential anticancer effect of quercetin on two HCC cell lines which differ on p53 expression, evaluate its effect on ^{18}F-FDG uptake and in GLUT-1 expression.
Methods: Two different HCC cell lines (HepG2 (wp53) and HuH7 (mp53)) were used. In order to assess the effect of quercetin in these cell lines, the cells were incubated in the presence of different concentrations of this compound for different periods of time, and after cell proliferation was evaluated by the MTT test in order to calculate half maximal inhibitory concentration (IC_{50}). The type of cell death was assessed by flow cytometry using the double staining with annexin-V and propidium iodide. Bax, Bcl2 and GLUT1 expression was also assessed by flow cytometry. For uptake studies, ^{18}F-FDG was incubated in a cell suspension in cells pre-incubated with quercetin and control cells. At different times, samples were collected to eppendorf tubes for uptake calculation. Eppendorfs were then centrifuged and radioactivity of pellets and supernatants was measured with a well-type gamma counter.
Results: Quercetin inhibits cell proliferation in HepG2 and HuH7 cell lines in a time-dependent manner. This compound does not inhibit GLUT1 expression, however is able to decrease the ^{18}F-FDG uptake in both cell lines. Flow cytometry results have shown that quercetin has a cytotoxic effect only at high concentrations of this compound. When cell death occurs, is mainly by apoptosis and this is accompanied by an Bax activation.
Conclusion: This study showed that quercetin has a considerable anti-proliferative effect in HepG2 and Huh7 cell lines. This compound probably modifies the function but not the expression of GLUT1, since it inhibits ^{18}F-FDG (a glucose analogue that is transported into

the cell by GLUT1 and GLUT3) uptake. In this context quercetin may represent a new therapeutic option in HCC.

P32. NEW PERSPECTIVES ON PRIMARY LIVER TUMORS DIAGNOSIS: THE ROLE OF ^{18}F-FDG AND ^{18}F-FLUOROCHOLINE

A.F. Brito, M. Ribeiro, A.M. Abrantes, F. Castro-Sousa, J.G. Tralhão, M.F. Botelho

Biophysics Unit, IBILI - Faculty of Medicine, University of Coimbra, Coimbra; Center of Investigation on Environmental, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra; Faculty of Sciences and Technology, University of Coimbra, Coimbra; Surgical Department, Surgery A, HUC, Coimbra.

Introduction: The incidence of primary liver tumors (PLT) has increased in recent years, especially in developing countries. Hepatocellular carcinoma (HCC) is the most common PLT (80%) followed by cholangiocarcinoma (CaC) (10%). A major difficulty is to obtain an accurate diagnosis that allows also distinguishing between PLTs. The aim of this work is to study the uptake profile of ^{18}F-FDG and ^{18}F-Fluorocholine in two PLT cell lines a HCC cell line and a CaC cell line.
Methods: The cell lines used were HuH7 (HCC) and TFK1 (CaC). ^{18}F-FDG and ^{18}F-Fluorocholine were incubated in a cell suspension with 2×10^6 cells/ml (25 µCi/ml). Samples of 200 µl were collected to eppendorf tubes for tracer uptake determination. Eppendorfs were then centrifuged and radioactivity of cell pellets and supernatants was measured with a well-type gamma counter.
Results: We observed, in both cell lines, a higher ^{18}F-Fluorocholine uptake than ^{18}F-FDG uptake. However, it was found that CaC cell line has a higher uptake of both tracers than HCC cell line. After 120 minutes with radiopharmaceuticals incubation, the ^{18}F-FDG uptake by HCC cell line is about 1.6% and by CaC cell line is about 3%. For ^{18}F-Fluorocholine the uptake by HCC cell line is about 6% and by CaC cell line 30%.
Conclusion: These results show that both cell lines under study have higher ^{18}F-Fluorocholine than ^{18}F-FDG uptake, and CaC cell line has a higher uptake of both radiopharmaceuticals than HCC cell line. Although the results are not very satisfactory for HCC, in the case of CaC there is an increased uptake, mainly, of ^{18}F-Fluorocholine. So,^{18}F-Fluorocholine may provide an option for the diagnosis of this pathology.

P33. EFFECT OF PROINFLAMMATORY CYTOKINES UPON BUTYRATE UPTAKE AND UPON ITS CELLULAR TARGETS IN INTESTINAL EPITHELIAL CELL LINES (IEC-6 AND CACO-2 CELLS)

T.A. Catarino, P. Gonçalves, F. Martel

Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Porto.

Introduction: Intestinal inflammation is known to predispose and to play a crucial role in the development of colorectal cancer (CRC), one of the most common cancers worldwide. The short-chain fatty acid butyrate (BT) plays a key role in colonic epithelium homeostasis, being able to prevent/inhibit colon carcinogenesis. BT is transported into colonic epithelial cells by two specific carrier-mediated transport systems, the monocarboxylate transporter 1 (MCT1) and the Na^+-coupled monocarboxylate transporter 1 (SMCT1), both being proposed to function as tumour suppressors. Recently, the proinflammatory cytokines tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were reported to decrease MCT1 and SMCT1 mRNA and protein levels. So, our aim was to characterize the effect of the proinflammatory cytokines TNF-α and IFN-γ upon (a) the uptake of BT in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines, and upon (b) the effects of BT on cell proliferation and viability.
Methods: The uptake of ^{14}C-BT was measured by liquid scintillometry, and the effects of the proinflammatory cytokines and BT on cell viability and proliferation were accessed with the lactate dehydrogenase assay and the sulforhodamine B assay, respectively.
Results: A 24h exposure to TNF-α and IFN-γ (100 ng/mL) resulted in a 15-25% inhibition of ^{14}C-BT uptake in IEC-6 cells, but this inhibition was associated with a marked decrease in cell viability and proliferation. In contrast, a 24h exposure of Caco-2 cells to TNF-α and IFN-γ (200 ng/mL) resulted in a ~10% inhibition of ^{14}C-BT uptake, without compromising cellular viability or proliferation. BT (10 mM; 24h) caused a significant reduction in Caco-2 cell proliferation and viability. Simultaneous treatment with IFN-γ (200 ng/mL; 24h) did not modify the effects of BT upon cell proliferation and viability. In contrast, simultaneous treatment with acetylsalicylic acid, a known anti-inflammatory molecule, potentiated the effect of BT upon Caco-2 cell proliferation.
Conclusion: The proinflammatory cytokines TNF-α and IFN-γ were able to cause a small inhibition of BT uptake in Caco-2 cells. Given the role played by BT in the intestine, this mechanism may contribute to the tumour-promoting effect of proinflammatory cytokines at this level.
Supported by Fundação para a Ciência e a Tecnologia (FCT) and COMPETE, QREN and FEDER (PTDC/SAU-OSM/102239/2008). The authors wish to thank Prof. Fernando Magro (Department of Pharmacology and Therapeutics, Faculty of Medicine of Porto, Portugal) for the generous gift of IFN-γ.

P34. EVALUATION OF THE POTENTIAL THERAPEUTIC EFFECT OF EPIGENETIC MODULATORS ON HEPATOCELLULAR CARCINOMA

L. Vieira, P. Silva, S. Neves, A.B. Sarmento-Ribeiro, J.M. Nascimento Costa

Faculty of Medicine, University of Coimbra (FMUC), Coimbra; Center of Investigation on Environment, Genetics and Oncobiology (CIMAGO), FMUC, Faculty of Medicine, University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; University Hospital of Coimbra (HUC/CHUC), Coimbra.

Introduction: Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer related deaths, with the heaviest burden on Southeast Asian and African countries, due to high rates of chronic Hepatitis B Virus (HBV) infection. The tumor usually presents in an advanced stage, when surgical resection is non-curative. Thus, research on new and more effective chemotherapeutic approaches is necessary. Besides the already known genetic mutations found on HCC cells, it has recently been accepted that epigenetic modifications may play a pivotal role on hepatocarcinogenesis. These mechanisms involve CpG islands methylation and histone deacetylation, altering the expression levels of several genes, namely tumor suppressor genes and proto-oncogenes. In opposite to genetic mutations, these mechanisms are reversible, so that they may be explored as new therapeutic approaches on hepatocellular carcinoma. In fact, drugs targeting these mechanisms are already available for the treatment of myelodysplastic syndromes.
Objectives: The aims of this work are to study the epigenetic modifications in cell cycle and apoptosis regulators related genes in hepatocellular carcinoma cell lines *in vitro* and the therapeutic potential of epigenetic modulators.
Methods: To attain these objectives we used 3 different hepatocellular carcinoma cell lines with different etiology and genetic alterations, namely on p53 expression, the HUH-7, Hep3B

and HepG2 cells. Methylation profile of cell cycle and apoptosis regulators genes, namely *p21, p15, p16, DAPK, PTEN, RASSF1* and *GSTP1*, were studied by a Methylation Specific PCR using specific primers. and performed before and after the treatment of cells with two epigenetic drugs, trichostatin (a histone deacethylase inhibitor) and decitabine (a hypomethylating drug). Additionally, the cell viability was evaluated using the Alamar Blue reduction assay at 24, 48 and 72 hours. The mechanisms involved in cell death were evaluated by flow cytometry using Annexin V/Propidium iodide.
Results: Our results show that *p21, p15, p16, DAPK, RASSF1 and GSTP1* genes have, in the different HCC cell lines, a differential methylated pattern. The epigenetic modulators Trichostatin (TSA) and Decitabine (DAC) induced a decrease in cell proliferation and viability in a dose, time and cell dependent manner. A synergetic effect was observed upon incubation of HCC cell lines with TSA and DAC simultaneously. This cytotoxic effect occurs mainly by apoptosis as assessed by flow cytometry. There is also evidence that epigenetic modifications can be reverted after incubation with epigenetic modulators.
Conclusion: This study reinforces the theory that epigenetic modifications are involved in hepatocarcinogenesis and shows that epigenetic modulators (epidrugs) may be useful as new therapeutic approach in hepatocellular carcinoma.

P35. DNA METHYLATION PROFILE IN PATIENTS WITH HEPATOCELLUAR CARCINOMA

P. Silva, S. Neves, L. Vieira, A.B. Sarmento-Ribeiro, J.M. Nascimento Costa

Faculty of Medicine, University of Coimbra (FMUC), Coimbra; Center of Investigation on Environment, Genetics and Oncobiology (CIMAGO), FMUC, Faculty of Medicine, University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra; University Hospital of Coimbra (HUC/CHUC), Coimbra, Portugal.

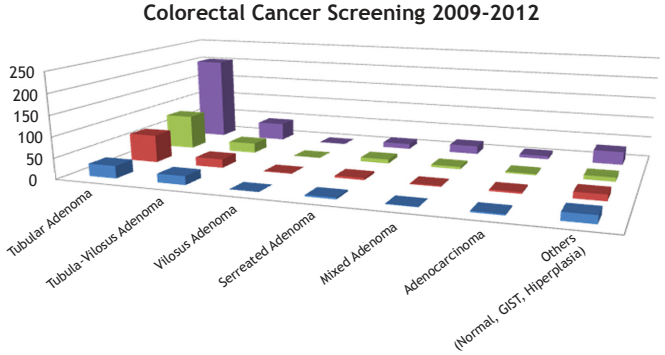
Introduction: Hepatocellular carcinoma (HCC) is one of the most common hepatic cancers. The tumor usually presents at an advanced stage, when surgical resection is non-curative. Besides the already known genetic mutations found on HCC cells, it has recently been accepted that epigenetic gene expression modifications may play a pivotal role on hepatocarcinogenesis. Furthermore, it has been shown that a variety of epigenetic alterations can cause molecular heterogeneity of liver tumors. These mechanisms involving CpG islands methylation and histone deacetylation, influenced the expression levels of several genes, namely tumor suppressor genes and proto-oncogenes. Aberrant methylation of gene promoters can be associated with tumor progression and etiological risk factors (HBV or HCV infection and alcohol consumption) and also correlated with survival after cancer therapy.
Methods: To further understand the molecular mechanism of hepatocarcinogenesis, we have investigated, in a pilot study, the gene promoter methylation status of cell cycle, apoptosis and adhesion regulators related genes, namely p21, p15, p16, p53, DAPK, SOCS-1, PTEN, RASS, GSTP, β-catenin and E-cadherin, in patients with HCC and tried to correlated the methylation profile with clinical and pathological patient's characteristics. The study was first performed on DNA extracted from paired fresh frozen tumor, adjacent normal tissues and blood samples from 15 patients using a methylation-specific polymerase chain reaction.
Results: Our results show that patient's tumors with different etiologies show differential methylated gene patterns in blood samples as well as in the tumor and adjacent normal tissue. Furthermore, co-existence of methylated with unmethylated DNA, in some patient´s samples, suggested that both genetic and epigenetic mechanisms may act in concert to regulate gene expression in HCC.
Conclusion: This study reinforces the theory that epigenetic modifications are involved in hepatocarcinogenesis and better understanding of the global deregulation of methylation states and how they correlate with disease progression will aid in the design of strategies for earlier detection and better therapeutic decisions.

P36. COLORECTAL CANCER SCREENING 2009-2012

T. Ferreira, M.J. D'Aguiar, L. Carvalho

Institute of Anatomical Pathology, Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Screening is checking for problems before they cause symptoms. If colorectal cancer screening reveals a problem, diagnosis and treatment can occur promptly. Colorectal cancer is generally more treatable when it is found early, before it has a chance to spread.
Methods: Colorectal cancer screening started in Portugal in the year 2009 centered in the Institute of Anatomical Pathology – Faculty of Medicine of the University of Coimbra. There were analysed 649 cases presented in the graphic, concerning 372 patients.
Results: Shown in figure and table.



	Tubular Adenoma	Tubulo Vilosus Adenoma	Vilosus Adenoma	Serreated Adenoma	Mixed Adenoma	Adenocarci-noma	Others (Normal, GIST, Hiperplasia)
Nº Cases/Patients 2009 (78/43)	30	21	0	4	1	3	19
Nº Cases/Patients 2010 (115/56)	67	21	1	5	2	4	15
Nº Cases/Patients 2011 (136/73)	83	24	0	10	6	3	20
Nº Cases/Patients 2012 (320/200)	204	43	0	13	20	8	32

Conclusion: It is relevant that 372 patients had an incidence of 18 well differentiated adenocarcinomas, which equals 4.83%. The Tubular adenomas presented as the most prevalent histological pattern.

P37. BUTYRATE AS AN ANTI-CANCER AGENT IN THE PREVENTION OF COLORECTAL CANCER: A STUDY IN THREE COLORECTAL CANCER CELL LINES

J.E. Encarnação, T.J. Gonçalves, A.S. Pires, C.R. Marques, A.M. Abrantes, J.E. Casalta-Lopes, M.F. Botelho

Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra; Faculty of Sciences and Technology, University of Coimbra, Coimbra; Centre of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra.

Introduction: Colorectal cancer (CRC) is one of the cancers with higher incidence and simultaneously with a high mortality. An important diet based on high levels of dietary fiber is related with a lower risk for developing colon cancer. Microbial fermentation of dietary fiber by the gut results in production of short chain fatty acid (SCFA) such as butyrate, acetate and propionate. The butyrate is an important energy source for colonocytes (70-90%) and it plays an important role in maintenance of the colon homeostasis. It was also reported that butyrate may be able to be a chemopreventive agent, being capable to inhibit the angiogenesis and HDAC's, increase TGF-β-induced Smad protein phosphorilation, and increase 15-hydroxyprostagladin activity. The aim of this study is to evaluate the effect of butyrate on the proliferation of three cancer cell lines (WiDr, LS1034, C2BBe1- ATCC) in order to determine the respective IC50 (half maximal inhibitory concentration).
Methods: WiDr (colon) and C2BBe1 (colon) cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) whereas LS1034 (cecum) cells were cultured in RPMI supplemented with 10% FBS. For each experiment, cells were plated in 48 multiwells in a concentration of 50,000 cells/mL. After 24 hours, cells were incubated with different sodium butyrate concentrations (1-50 mM). In order to determinate the IC50 after 24, 48, 72 and 96 hours, the cell proliferation was evaluated through MTT assay.
Results: In all three cell lines, 24 hours after the treatment with butyrate, the results showed a slight decrease of cell proliferation with increasing doses of butyrate. However, it was observed that when cells are subjected to butyrate for a longer time (48h, 72h and 96h) cell proliferation decrease with low IC50 values. The results are similar in the three cell lines decreasing the IC50 value with butyrate incubation time.
Conclusion: Our study suggests that butyrate has an anti-proliferative effect on the three CRC cell lines despite of the different genetic background and organ localization.

P38. SYNTHESIS AND CHARACTERIZATION OF 5, 10, 15, 20-TETRAKIS-(4-CARBOXYPHENYL)-PORPHYRIN (TCPP) FOR PHOTODYNAMIC THERAPY: PHOTOSENSITIVE POTENTIAL IN THE COLON CANCER CELL LINE HT29

L. Costa, J.A. Silva, C.T. Arranja, M. Marcos, A.M. Urbano, AJFN Sobral

Unidade de Química-Física Molecular and Departamento de Ciências da Vida, FCT, Universidade de Coimbra, Coimbra; Departamento de Química, FCT, Universidade de Coimbra, Coimbra; CIMAGO, Faculdade de Medicina, Universidade de Coimbra, Coimbra.

Introduction: Photodynamic therapy (PDT) is a technique with great potential in oncological therapy. This therapeutic strategy is based on the administration of a photosensitiser, which is selectively retained in tumour tissues, followed by irradiation of the tumour with visible light in a wavelength range matching the absorption spectrum of the photosensitiser. The absorption of light by the photosensitiser induces the production of reactive oxygen species (ROS), generating a cascade of events that kills the tumour cells. The characteristics of porphyrins make them especially suited as photosensitizers. This study describes the synthesis of 5, 10, 15, 20-tetrakis-(4-carboxyphenyl)-porphyrin (TCPP) and its characterization as a sensitizer for PDT.
Methods: After synthesis, chemical and photo-physical characterization, the photodynamic effect of TCPP was evaluated in HT29 cell cultures. Briefly, HT29 cultures were incubated with the sensitizer (2.5–20 μg/mL, final concentration) in DMSO solution (0.5% v/v). The cultures were irradiated during 0 to 30 min, 24 h after sensitizer addition. Cell viability was evaluated by the MTT assay, 24 h after irradiation of the cell cultures. For each experiment, porphyrin-treated cultures and control cultures (treated with DMSO only) were processed in parallel.
Results: TCPP showed photo-physical characteristics of great interest for PDT, namely high singlet oxygen quantum yield and good absorption between 600 and 800 nm. Additionally, TCPP showed no cytotoxicity in the absence of light. On the contrary, after activation by light, TCPP induced a significant decrease in cell viability.
Conclusion: Upon photodynamic treatment, TCPP had the capacity to decrease the viability of HT29 cells. In view of a possible clinical use, further studies will be performed aiming at the elucidation of the intracellular localization of TCPP and the mechanism by which it kills cancer cells.
Acknowledgements: This work was supported by Fundo Europeu de Desenvolvimento Regional - QREN - COMPETE (projects PTDC/AAC-CLI/098308/2008 and PTDC/AAC-CLI/118092/2010 of Fundação para a Ciência e Tecnologia (FCT) to AJFNS) and grant PEst-OE/QUI/UI0070/2011, to Unidade de Química Física Molecular. The authors thank Dr F. Gil (Departamento de Física, Universidade de Coimbra) for use of the power meter.

P39. A NEW APPROACH AGAINST HEPATOCELLULAR CARCINOMA: STATINS

V. Quintã, J. Balteiro, V. Rodrigues

Department of Pharmacy, College of Health Technology of Coimbra (ESTeSC), Polytechnic Institute of Coimbra (IPC), Coimbra.

Introduction: Hepatocellular carcinoma (HCC), one of most common causes of cancer death worldwide, is characterized by a poor prognosis and recurrence within a short time. The available antineoplastic chemotherapy is highly toxic to patients and is becoming increasingly inefficient due to the resistance developed by tumor cells. Thus in an attempt to find new alternatives, it was discovered that statins, inhibitors of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR), have potential as antineoplastic drugs. Researchers think that this occurs because statins block the mevalonate pathway, preventing the production of non-steroid isoprenoids, important for the modification and function of small GTPases in cell growth, angiogenesis, cell migration and cell invasion. Thereby, this study focuses on some of the newly discovered targeted therapies of statins in HCC cells, describing briefly the mevalonate pathway, and presenting some limitations of the use of statins and some of their synergistic associations with other drugs identified for anticancer purposes.
Methods: It was conducted a search in PubMed and ScienceDirect to obtain articles, preferably original, published between 2008 and 2012, using keywords such as statins, hepatocellular carcinoma, associations and apoptosis, selecting articles by title and abstract.

Thereafter, the resulting articles were read in full, being made a second selection of the articles of interest to this study.
Results: Following the previous reasoning, through the blocking of mevalonate pathway, there were recently identified some of statins' biological targets, in HCC cells: decrease of integrin expression and blockade of Rho/ROCK pathway; regulation of cyclin-dependent kinases (CDK), cyclins and CDK inhibitors; and, inhibition of thioredoxin reductase 1 (TrxR1) expression. However, the use of these drugs for anticancer purposes has some limitations, such as HMGCR's regulation by negative feedback, statins' biphasic effects on angiogenesis, statins' side effects and toxicity, and statins' drug interactions. Therefore, researchers associated statins with other drugs to overcome these limitations, for instance geraniol, celecoxib, protein kinase C (PKC) inhibitors and natural products.
Conclusion: In the future, to develop statins as antineoplastic agents, it would be important to: identify molecular markers predictive of response; identify adequate pharmacodynamic parameters to guide dose escalation for their administration and help to assess the response to them; perform clinical trials for a benefit/risk evaluation of their use in cancer; and, study in which situations their use represents an added value comparatively to the traditional chemotherapy.

P40. THE ROLE OF ADIPOCITOKINES IN COLORECTAL CANCER DEVELOPMENT AND PROGRESSION – A PRELIMINARY STUDY

A. Pereira, D. Silva, P. Matafome, A.C. Gonçalves, R. Seiça, A.B. Sarmento-Ribeiro, J. Nascimento-Costa

Medicine Service, Hospital Distrital da Figueira da Foz (HDFF, EPE), Figueira da Foz; CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, FMUC, Coimbra; Physiology, FMUC - Faculty of Medicine University of Coimbra, Coimbra; IBILI - Institute of Biomedical Research in Light and Image, FMUC, Coimbra; Applied Molecular Biology and University Clinic of Hematology, FMUC - Faculty of Medicine University of Coimbra, Coimbra; Center for Neuroscience and Cell Biology (CNC); Medicine Department, Centro Hospitalar e Universitário de Coimbra (HUC/CHUC), Coimbra; Oncology and Paliative Care, FMUC, Coimbra.

Introduction: Colorectal cancer (CCR) is one of the most frequent types of cancer in the western world and obesity has been associated with increased incidence of this cancer. One of the mechanisms proposed to contribute to cancer pathogenesis and progression, namely in CCR, is adipose tissue dysfunction, in particularly alterations in adipocytokines levels like resistin, leptin or visfatin. The knowledge of the pathophysiological mechanisms of the various signals transductions pathways underlying the association between obesity and cancer, may be important for the better understanding of the development and progression of this neoplasia. Besides that these knowledge may contribute to identify new prognostic markers and therapeutic approaches.
Objectives: The aim of this study was to investigate the association between several blood adipocytokine levels and the clinicopathological characteristics of CCR patients, and its possible role in the development and progression of CCR.
Methods: This study enrolled 39 CCR patients (13F/26M) with a median aged of 72 (41 a 91) years, being 62% rectal adenocarcinoma and 38% colon adenocarcinoma. According to TMN staging, 11% of these patients were in stage I, 36% in stage II, 39% in stage III and 14% in stage IV. We also study 18 healthy controls (7F/11M) with a median aged of 66 (48-85) years. The levels of the adipocytokines, adiponectin, leptin, resistin, visfatin, TNF-α and MCP-1, were tested in serum samples using Elisa commercial kits.
Results: Ours results show that CCR patients with excessive body weight presents higher resistin (23 ± 20,6 ng/ml), visfatin (124 ± 95 pg/ml) and MCP-1 (735 ± 335 ng/ml) levels and lower adiponectin levels (5.3 ± 2.8 µg/ml) when compared to CCR patients with normal body weight (20,6 ± 12.1 ng/ml; 83.2 ± 33.2 pg/ml; 410 ± 95 ng/ml; 8.1 ± 5.4 µg/ml, respectively) and controls (13.2 ± 10.2 ng/ml; 73.8 ± 62.5 pg/ml; 508 ± 165 ng/ml; 5.8 ± 3.5 µg/ml, respectively). We also observed changes in MCP-1 levels according with tumor localization, being statistically different in ascending colon (463 ± 171 ng/ml) compared with sigmoide colon (81 ± 41 ng/ml). Moreover, the visfatin and leptin levels may be related with progression of CCR, since we observed statistical differences between patients in stage III (leptin 10.1 ± 6.0 ng/ml; visfatin 115 ± 76 pg/ml) compared with those in stage IV (leptin 5.78 ± 0.12 ng/ml; visfatin 52.5 ± 2.7 pg/ml).
Conclusion: This preliminary results suggest that adipose tissue dysfunction, in particular alterations in adipocitokines levels, may be involved in development and progression of CCR.
This work was supported by CIMAGO (Project 25/09).

HEAD AND NECK CANCER

P41. FROM GENOME-WIDE PROFILING OF ORAL CAVITY CARCINOMA TO MOLECULAR TARGETED THERAPIES... HOW FAR ARE WE?

I.P. Ribeiro, F. Marques, S.I. Ferreira, J. Ferrão, A. Cainço, M.J. Julião, I.P. Baptista, J.B. Melo, I.M. Carreira

Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia; Area de Medicina Dentária, Faculdade de Medicina, Universidade de Coimbra, Coimbra; Serviço de Anatomia Patológica, Hospitais da Universidade de Coimbra, HUC, EPE, Coimbra.

Introduction: Head and neck cancer (HNC) encompasses a diverse range of tumors that include the oral cavity, pharynx and larynx. Worldwide, almost 635,000 new cases are diagnosed annually and 358 000 people die from HNC each year (Ferlay et al. Int J Cancer. 2010;127(12):2893-917). Although these tumors display a great genetic heterogeneity with alterations in almost all chromosomes until now only few genes have been associated with oral carcinogenesis process. Recent developments have focused on the use of agents that target the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor receptor (VEGFR). Therefore, nowadays, from the genetic imbalances already identified it is difficult to understand which of those can be used individually or combined, in order to routinely predict the clinical outcome of the oral tumors. Thus, the clinical choice of treatment is based on specific tumor site, stage of the disease and pathological findings.
Objectives: Taking this in mind, the main goal of the present study was the identification of the genomic profile of oral tumors from patients with oral cavity carcinoma diagnosis, through the application of whole genome array-Comparative Genomic Hybridization (aCGH) as well as Multiplex Ligation-dependent Probe Amplification (MLPA) technique.
Methods: Biopsies of tumor were acquired from 28 patients. The aCGH was performed in tumor samples using an Agilent oligonucleotide microarray 4x180K and the MLPA was conducted using 4 MLPA probe panels specific for tumor samples. Healthy donors were used as controls.
Results: In general, we identified 8,815 genes with loss and 1,409 genes with gain. Although we detected alterations in the great

majority of the chromosomes, it was possible to observe that the most commonly losses and gains were detected in specific chromosomal regions. The sizes of the imbalances are very different between patients. Despite this heterogeneity it is possible to observe a pattern of alterations in some chromosomes and also a great number of patients with the same chromosomal regions/genes altered. These results highlight some genes with strong possibility to be key genes in the oral carcinogenesis.
Conclusion: We detected alterations in the great majority of the chromosomes, however, it was possible to observe a pattern of common alterations. Regarding these imbalances, which include specific and discriminative genes, novel therapeutic targets to oral cancer can be developed. Thus, this study underlines some putative new biomarkers with possible diagnostic and prognostic value.

P42. REMOVED BY THE AUTHORS

P43. HPV IN THE ETIOLOGY OF ORAL CAVITY CARCINOMA: TRUE OR COINCIDENCE?

I.P. Ribeiro, F. Marques, H. Prazeres, J. Ferrão, M.J. Julião, I.P. Baptista, J.B. Melo, I.M. Carreira

Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia; Area de Medicina Dentária, Faculdade de Medicina, Universidade de Coimbra, Coimbra; Laboratório de Patologia Molecular do IPO de Coimbra FG, EPE, Coimbra; Serviço de Anatomia Patológica, Hospitais da Universidade de Coimbra, HUC, EPE, Coimbra.

Introduction: Tobacco, alcohol and human papillomavirus (HPV) are now well-established risk factors for head and neck cancer (HNC), which includes the oral cavity carcinoma. In 2010, Ramqvist and Dalianis (Ramqvist et al. Emerg Infect Dis. 2010;16(11):1671-7) classified these tumors as the second most common HPV-associated cancer. These cancers are distinct epidemiologically, clinically, and molecularly from HPV-negative tumors and are associated with improved survival outcomes (Fakhry et al. J Natl Cancer Inst. 2008;100(4):261-9). In general, HNC patients with HPV-positive are younger and without the traditional risk factors of smoking and alcohol consumption (Marur et al. Lancet Oncol. 2010;11(8):781-9). Since HPV is associated not only with cervical cancer and genital warts but also with other tumors, such as HNC, the implementation of vaccination programmers also for men remains as a challenging issue. The main goal of the present study was to detect and typify HPV in patients with oral cavity carcinoma diagnosis and to analyze a possible correlation between HPV and the genomic profile.
Methods: This study was conducted on 28 oral tumor samples, which only have 2 women in the cohort. All samples were screened for HPV infection by PCR using the degenerate consensus primers MY09/11 and GP5+/6+. The identification of the genomic profile of these tumors was performed using Multiplex Ligation-dependent Probe Amplification technique.
Results: We detected HPV positive in 2 patients. The types of HPV in these 2 patients are different, one type is considered as low-risk (type 42) and the other is high-risk (type 31). Both patients exhibit oral tumors in advanced stage (IVa), but only the patient with low-risk HPV do not have other risk factors (tobacco, alcohol). Regarding the molecular genomic profile, these patients shown few genetic imbalances. However, only with 2 patients it is very difficult to clarify if patients with HPV positive represent one distinct HNC group with a genetic profile completely different from the others patients with tobacco/alcohol risk factors.
Conclusion: The HPV detection in HNC patients is important in order to help the prediction of treatment outcome. It is mandatory further studies in order to understand which types of HPV are more frequent in oral cavity carcinoma patients. Taking into account that incidence of these tumors has been increased mostly in younger people, HPV vaccines seems an attractive choice and justify deep reflections. This study supports the notion that men can be an important target to HPV vaccination in the case of oral tumors.

P44. CAN A ORAL LEUKOPLAKIA EVOLVE TO A TUMOR: WHAT GENES CAN TELL US?

I.P. Ribeiro, F. Marques, J. Ferrão, M.J. Julião, J.B. Melo, I.P. Baptista, I.M. Carreira

Laboratório de Citogenética e Genómica, Faculdade de Medicina, Universidade de Coimbra, Coimbra; CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia; Area de Medicina Dentária, Faculdade de Medicina da Universidade de Coimbra, Coimbra; Serviço de Anatomia Patológica, Hospitais da Universidade de Coimbra, HUC, EPE, Coimbra.

Introduction: Oral cancer is considered to be the final stage of a multistep process evolving from normal histology to hyperplasia, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma *in situ*, and invasive carcinoma. The term "oral potentially malignant disorders" was recommended by an international working group convened by the WHO Collaborating Centre for Oral Cancer and Pre-Cancer in London, in 2005. It is believed that not all these disorders will evolve to invasive cancer, at least not within the lifetime of these patients. Leukoplakia represents one of the most common potentially malignant oral lesions. The term leukoplakia should be used to recognize white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer. The main goal of this study was the identification of the genetic profile of an oral tumor and a leukoplakia.
Methods: We identified the genetic profile of one tumor of the oral cavity as well as of a leukoplakia, both from the same patient. The leukoplakia was identified and biopsied at the same time of the tumor diagnosed. The genetic study was conducted using the Multiplex Ligation-dependent Probe Amplification technique.
Results: Taking into account the clinical type, the present leukoplakia was recognized macroscopically as being non-homogeneous, which carry a much higher risk of malignant transformation. Among the different varieties of non homogeneous leukoplakia, the lesion in study includes speckled: mixed, white and red, but retaining predominantly white character. Regarding the genetic profile of tumor, we identified gains in some genes of the long arm of the chromosome 8 and 11. In the leukoplakia we observed gains especially in the 11q13, where are mapped the *CCND1, FGF3* and *CTTN* genes. The amplification of chromosomal region 11q13 is a common imbalance in oral tumors, and seems to be correlated with unfavorable outcomes.
Conclusion: In this patient the tumor presented more genetic imbalances than in leukoplakia, nonetheless some specific genes altered in tumor were altered in leukoplakia. Both leukoplakia and tumor share the same genetic profile in some regions, which can be indicative of the malignant transformation of this lesion and consequently of the risk of relapse. It is generally accepted that screening of oral potentially malignant lesions may decrease the great morbidity and mortality of oral cancer. Thus, this case study reinforces the importance of performing the genetic follow up of suspicious leukoplakia in order to better evaluate the risk of malignancy.

PROSTATE CANCER

P45. DEVELOPMENT OF AN ORTHOTOPIC MODEL OF PROSTATE CANCER: A STEP CLOSER TO THE REAL IN VIVO MODEL

A.C. Mamede, E. Tavares-Silva, A.M. Abrantes, F. Botas, A.S. Pires, A.C. Gonçalves, A.B. Sarmento-Ribeiro, P. Simões, A. Mota, C.J. Maia, M.F. Botelho

Biophysics Unit, Institute for Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra, Coimbra; CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Covilhã; Centre of Investigation on Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra; Urology and Transplantation Department, Coimbra Hospital and University Centre, Coimbra; Faculty of Medicine, University of Coimbra, Coimbra; Faculty of Sciences and Technology, University of Coimbra, Coimbra; Applied Molecular Biology and Hematology Group, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Prostate cancer (PCa) is one of the most frequently diagnosed cancers. Despite prevention, diagnosis and therapeutic options currently available, it is not rare to find that some apparently localized tumors progress to metastatic disease, or are already metastatic at diagnosis. Thus, the development of an animal model capable to reliably reproduce PCa behavior can be an understanding key to study the mechanisms involved in its progression, thereby contributing to a better planning of prevention, diagnosis and therapy strategies. Orthotopic transplantation of human tumor cells, compared to heterotopic option, seems to be an alternative that leads to the development of PCa in animals, with similar expression of human cancer clinical features.
Methods: Studies were performed in PCa cell lines obtained in ATCC: LNCaP (androgen/estrogen dependent) and PC3 (androgen/estrogen independent). Hormonal profile was confirmed through flow cytometry, as well as Her2/neu expression. Ki-67 labelling index was determined *in vitro* to compare cell proliferation between LNCaP and PC3. For orthotopic inoculation of both PCa cell lines, RNU immunosuppressed male rats were used. After anesthesia, animals underwent surgery in order to inoculate 15×10^6 cells/animal in the dorso-lateral portion of the prostate gland. It was performed a daily behavioral evaluation of the rats and a weekly body weight measurement.
Results: As described by ATCC, LNCaP cell line expresses the androgen and estrogen receptor. Moreover, PC3 cell line does not express both receptors. Expression of Her2/neu is four times higher in hormone-dependent LNCaP than in the hormone-independent PC3 cell line. Ki-67 labelling index is higher in PC3 cell line. As described in literature, it is difficult to obtain a metastatic PCa rat model. Death usually occurs due to locally advanced disease, prior to the metastatic disease. Some of our specimens survived several months after PCa implantation. On exploratory laparotomy we have seen that the tumor occupies the entire gland, protrudes to the bladder, reduces its functional capacity, but does not compromise urinary drainage at this time. However, there is no evidence of metastasis.
Conclusion: LNCaP and PC3 cell lines have distinct hormonal profiles and Her2/neu expression. PC3 presents a greater proliferative rate, according to Ki-67 labeling index. According to preliminary results regarding the orthotopic model, it is rational to perform a urinary diversion on rat, in order to avoid the complications of locally advanced disease, allowing it to reach the metastatic PCa phase.

P46. PT(II) AND PD(II) POLYAMINE COMPLEXES AS PROMISING ANTICANCER APPROACHES TOWARDS METASTATIC PROSTATE CANCER

C.S. Matos, A.L.M. Batista de Carvalho, M.P.M. Marques

Molecular Physical Chemistry R&D Unit, University of Coimbra, Coimbra; Departament of Life Sciences, Faculty of Science and Technology, University of Coimbra, Coimbra.

Introduction: Prostate cancer is the second most common cancer worldwide and the sixth cause of cancer-related deaths in men. When hormone therapy fails to control tumour growth, hormone refractory prostate cancer (HRPC) occurs and chemotherapy drugs must be administered. Despite the former belief that platinum drugs have a modest activity against prostate cancer, these agents have recently been the object of a growing interest, in particular in combination regimens. Cisplatin and its analogues are commonly used in a variety of cancers. However, they induce several deleterious side-effects and acquired resistance, which often limits their clinical use. Hence, new strategies are needed in order to overcome these injurious side-effects, namely the development of new drugs with a lower toxicity and an optimised cytotoxic profile.
Methods: Multinuclear polyamine complexes differing in the nature of the metal centre (Pt(II) or Pd(II)) as well as in the characteristics of the alkylamine ligands (spermine or spermidine) were investigated in this study, regarding their antineoplastic effect towards two human prostate cell lines: PC-3, derived from advanced androgen independent bone metastasis with a high metastatic potential, and PNT2, a human non-tumorigenic prostate cell line. The antiproliferative activity of these chelates was evaluated through the sulforhodamine-B (SRB) assay, while their cytotoxicity was assessed by the mitochondrial dehydrogenase activity MTT colorimetric test. Concentrations ranging from 5 to 100 μM were screened, for incubation periods of up to 72 hours. The reversibility of the drug effect was also determined, 72 h after removal of the complex-containing medium by by fresh, drug-free, medium.
Results: After an initial screening of the complexes, it may be concluded that the Pd(II) compounds are more effective than their Pt(II) analogues. When compared with cisplatin, the Pd_2-Spm chelate presented a higher cytotoxic effect coupled to a lower toxicity. In fact, the effect of this agent towards the non-neoplastic cells PNT2 was found to be reversed *ca.* 3 days after drug removal.
Conclusion: In the light of the gathered results, it may be concluded that Pd_2-Spm displays a promising antiproliferative and cytotoxic activity towards human metastatic prostate cancer, being even more effective than cisplatin.

P47. REMOVED BY THE AUTHORS

P48. REMOVED BY THE AUTHORS

LUNG CANCER

P49. CANCER STEM CELLS IN LUNG CANCER: THE RESIDENT EVIL!

C.F.D. Rodrigues, S. Ferreira, A. Paiva, F. Botelho, I.M.C. Carreira, M.C. Alpoim

PDBEB Programme, CNC, Universidade de Coimbra, Coimbra; CIMAGO, Universidade de Coimbra, Coimbra; CNC, Universidade de Coimbra, Coimbra; Faculdade de Medicina, Universidade de Coimbra, Coimbra; Centro de Histocompatibilidade, Hospitais da Universidade de Coimbra, Coimbra; Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra.

Introduction: Bypassing all the research advances in de last decades, cancer remains as a major public health. Recent research emphasized the major role of cancer stem cells (CSCs) in the metastatic disease, the main cause of cancer patients' mortality. CSCs drive tumorigenesis and differentiation, contributing to tumors' heterogeneity, resistance and eventually relapse. It was recently observed that they can emerge through dedifferentiation of other cells, although through a process still uncovered.
Methods and results: The non-malignant human bronchial epithelial cell line BEAS-2B was malignantly transformed into the RenG2 system using low density culture in the presence of hexavalent chromium [Cr(VI)]. A parallel control cell line (Cont1) was produced under the same conditions, though, in the absence of Cr(VI). Two additional derivative cell lines (DRenG2 and DDRenG2) were attained following serial rounds of injection in mice. Metabolic studies using [^{18}F]fluoro-2-deoxyglucose) and nuclear magnetic resonance spectroscopy performed in all the cell lines revealed a more glycolytic phenotype for the derivatives, compatible with a quiescent phenotype. Subsequent karyotype and real time PCR-based cellular characterization identified different cellular sub-populations within each cell line, strengthening the hypothesis on the CSCs presence. The sphere-formation assay confirmed the presence of CSCs only in the derivative cell lines, suggesting that a dedifferentiation process featured the formation of CSCs. The involvement of mice stroma in this process was uncovered by surgical isolation of mouse stromal cells from the subcutaneous compartment and subsequent co-culture with RenG2 cells for 30-60 days, which resulted in the emergence of a CSCs sub-population. Comparative genome hybridization array (aCGH) analysis performed in all the cell lines under study revealed a panel of potential paracrine orchestrators of this stromal-induced dedifferentiation process.
Conclusion: Tumor-associated stromal cells are able to induce the dedifferentiation of CSCs within a growing tumor, boosting its aggressiveness. Moreover, this process is chemically-based and specie-unspecific, once mice cytokines were able to act over human cells.
Aknoledgments: CIMAGO project 16/06 and FCT fellowship SFRH/BD/33884/2009.

P50. EFFECTS OF X RADIATION IN NON SMALL CELL LUNG CANCER – AN *IN VITRO* STUDY

R. Silva, F. Mendes, A.R. Guedes, A.M. Abrantes, A.C. Gonçalves, M. Laranjo, J. Casalta, P. César, P. Soares, A.B. Ribeiro, M.F. Botelho

Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra; Faculty of Science and Technology, University of Coimbra, Coimbra; Clinical Analysis and Public Health Department, College of Health Technology of Coimbra, Coimbra; Center for Research on Environment Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra; Center f or Neuroscience and Cell Biology, University of Coimbra, Coimbra; Applied Molecular Biology, University of Hematology Clinic Faculty of Medicine, University of Coimbra, Coimbra; Radiotherapy Service, HospitalCenter and University of Coimbra, Coimbra.

Introduction: Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases and include three sub-types: adenocarcinoma, large cell carcinoma and squamous cell carcinoma. This cancer develops resistance to radiotherapy and chemotherapy, being diagnosed usually in inoperable stages. The low sensitivity to radiation of NSCLC is one of the main reasons for the failure of radiotherapy. Exposure of the cells to ionizing radiation (IR) results in damage to cellular organelles, membranes and biomolecules by direct and indirect actions, which may lead to different results in different tissues and cell types. The lethal effects of IR are mainly due to DNA damage, and, indirectly, with stress oxidative generated in the cell. After the aggression, the cell can activate a number of signaling pathways, which may result in cell cycle arrest, activation of repair pathways or inducing death.
Objectives: To evaluate the effects of radiation X (RX) in monotherapy and/or in combination with chemotherapy in NSCLC cell lines viability and proliferation, and to characterize some of the mechanisms involved in cell death and cell cycle.
Methods: Two NSCLC cell lines with different p53 status, the H1299 ($p53^{null}$) and A549 cells ($p53^{WT}$), were used, in the absence (control) and after exposure to RX 4MeV with different doses (0.5 Gy, 15 Gy and 30 Gy), alone and in combination with the IC_{50} and IC_{25} of cisplatin and etoposide, every 24h for a period of 96h. Cell proliferation and viability were evaluated by Alamar Blue and clonogenic assay. Cell cycle and death were analyzed 48h after irradiation by flow cytometry using a double staining with annexinV/propidium iodide and PI/RNase, respectively.
Results and conclusion: Preliminary results suggest that RX induces a cytostatic and cytotoxic effect on both NSCLC cell lines in a dose, time and cell line dependent manner. Exposure to 15 Gy and 30 Gy induces a decreased in A549 cells proliferation, either alone or in combination with the chemotherapeutic drugs, inducing apoptosis. These results are consistent with the increase of the pre-apoptotic peak (pre-G0/G1) with increasing radiation dose, after 48h. In H1299 cells, we observe mainly a decrease in clonogenic potential, either in monotherapy or in combined therapy. After 48h irradiation there were no significant effects in cell proliferation, resting the majority of cell population in G2/M phase after irradiation with 15 Gy and 30 Gy and in S phase after combined therapy. This can be explained by the loss of checkpoint control by p53, which is absent in this cell line.

P51. BRACHYTHERAPY IN LUNG TUMORS

C. Fardilha, D. Roda, F. Costa, R. Vale, G. Melo

Serviço de Radioterapia, Instituto Português de Oncologia Francisco Gentil, E.P.E., Coimbra.

Introduction: Worldwide, lung cancer is the most common and the deadliest form of cancer. Surgical resection is the treatment

of choice, but only one third of patients who present with early disease are eligible for a curative resection. Brachytherapy is a form of radiation therapy delivered by the direct placement of a radioactive source into a tumour or tumour bed. Brachytherapy has the advantage of delivering a high dose to the tumor while sparing the surrounding normal tissues.
Objectives: Review into the current knowledge of lung brachytherapy, techniques and indications.
Methods: We selected several reference articles published in recent years related to the topic. Search reference books.
Results and conclusion: Brachytherapy is effective and has a good curative potential in patients with small to moderate size, well defined and easily accessible tumors. It can also be used to palliation in selected cases.

P52. *EGFR*, *HER2*, *ALK*, *MET* AND *KRAS* IN BRONCHIAL-PULMONARY ADENOCARCINOMAS TARGETED THERAPY

M.R. Silva, A. Alarcão, A.F. Ladeirinha, T. Ferreira, M.J. D'Aguiar, V. Sousa, L. Carvalho

Institute of Anatomical Pathology, Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO - Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Bronchial-pulmonary carcinomas develop as a multistep process linked to several intracellular pathways and several genetic alterations. The treatment and diagnosis of lung cancer has been revolutionized by targeted agents against genetic mutations. The identification of reliable predictive factors allows the selection of patients most likely to benefit from a particular agent and save others from toxicity of ineffective treatments. Identification of specific mutations: *EGFR, HER2, ALK, MET* and *KRAS* directs use of approved targeted therapies provide clinical benefit. Routine genetic testing for somatic mutations in lung cancer biopsies is the standard for proving optimal patient care.
Methods: A series of 62 adenocarcinomas classified in biopsies were tested according with cancer cells availability in the paraffin block. The presence of lung cancer cells was verified by pathological examination in hematoxylin-eosin sections, to apply FISH analysis and manual microdissection for *EGFR* and *KRAS* mutation analysis. Sanger sequencing of dissected lung cancer samples was performed to detect mutations in *EGFR* and *KRAS*. DNA was PCR amplified for exons 19 and 21 of *EGFR* and codons 12 and 13 of *KRAS*. *EGFR, ALK, HER2,* and *MET* analysis by FISH using dual-color probes was performed on paraffin-embedded tissue sections.
Results: Thirty two cases (33/62, 53%) of these samples had at least one of the targeted genetic alterations. EGFR exon 19 and 21 were mutated in 18/61; 5/41 exclusive cases had KRAS cases; 12/36 cases had EGFR FISH amplification; 4/32 cases had ALK fusion gene; 3/12 cases had MET amplification and 4/12 cases had HER2 amplification.
Conclusion: Supposing that heterogeneity of bronchial-pulmonary carcinomas is not as relevant previously understood, potential crosstalk between known pathways, used for a single targeted agent is the optimal strategy to substantially improve clinical outcome and multiple inhibition of intracellular pathways might offer an additional clinical benefit. By so our laboratory offers the chance to identify various possible targets that can best provide clinical benefit in the NSCLC setting. In the total 62 cases of diagnostic adenocarcinomas we determined and characterized 99% of these tumors, finding mutations in 53% of the samples with that will likely prove clinical benefit with targeted therapies.

P53. *P53* GENE IN THE CENTER REGION OF PORTUGUESE POPULATION WITH LUNG CANCER

P. Mota, A. Alarcão, J.R. Costa, J.P. Fonseca, A.F. Ladeirinha, T. Ferreira, M.J. D'Aguiar, L. Carvalho, F.J. Regateiro

Institute of Medical Genetics, Faculty of Medicine, University of Coimbra, Coimbra; Institute of Anatomical Pathology, Faculty of Medicine, University of Coimbra, Coimbra; Research Centre for Anthropology and Health, Faculty of Science and Technology, University of Coimbra, Coimbra; CIMAGO - Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: Lung cancer is one of the most common cancers in the world. Polymorphic genes like *p53,* involved in cell cycle regulation, apoptosis, and tumor suppression have been studied as possible risk factors for this cancer. To assess the role of putative mutated *p53* proteins in human lung cancer, we use monoclonal antibodies for immunohistochemistry assay.
Methods: We analyzed a possible relationship with demographic characteristics and clinical state of this population (n = 200), from HUC Hospitals, Coimbra, moreover with a *GSTT1* genotype association. *GSTT1* belong to a class of glutathione genes which play an important role as an antioxidant enzyme, involved in the primary cellular defense mechanism against reactive oxygen species. Individually, functional polymorphisms of these genes have also been studied as risk factors for lung cancer. DNA from peripheral blood samples was examined by RT-PCR for *GSTT1* polymorphism genotype (*GSTT1* Genotype: "Present":66.7%; "Null": 33.3%).
Results: We found associations between *p53* and some demographic variables such as tobacco habits (χ^2 = 8.973; *Sig.* = 0.011) and age ranges (χ^2 = 16.149; Sig. = 0.0001). When confronting *p53* results with clinical state of patients we found associations between this gene and tumor location (χ^2 = 4.717; Sig. = 0.030) as well as with age of diagnosis inside histology, namely in small cells lung cancer (SCLC) (χ^2 = 4.582; Sig. = 0.032, OD = 1.429, IC = [1.130;1.806]). *GSTT1* gene and *p53* mutated gene revealed a statistically significant association (χ^2 = 7.430; Sig. = 0.024).
Conclusion: The obtained results reinforced a relationship of bronchial-pulmonary carcinomas with smoking habits, for all histopathological types including small cell lung carcinomas.

P54. MESOTHELIOMA: INDICATIONS FOR RADIOTHERAPY

T.Q. Serra, D. Roda, M.P. Padilha, M. Henriques

Serviço de Radioterapia, IPO Coimbra FG EPE, Coimbra.

Introduction: Mesothelioma is a highly aggressive malignant tumor that usually has a fatal outcome that can arise in any pleural location. Incidence is growing and is closely related to asbestos exposure (80 to 90% of cases), predominantly in males (3-5:1) and advanced age (incidence peak in the 6th decade of life). The median survival for mesothelioma ranges between 9-17 months, with a 5 years survival rate that rarely exceeds 5%. The therapeutic approach of these tumors depends on a correct staging and resectability criteria that define which candidates are suitable to more aggressive therapeutic strategies. This work aims to identify the main indications for radiotherapy (prophylactic, as part of trimodality treatment or palliative) in mesothelioma.
Methods: Bibliographic search in MEDLINE databases, using the MeSH terms: Radiotherapy, Mesothelioma, Treatment, of articles published from January 2001 to December 2012 and in any language.
Results: The indications for radiotherapy in mesothelioma can be grouped into 3 categories: prophylactically to reduce the incidence of recurrence in locations subjected to surgical or diagnostic procedures, as part of the definitive locoregional therapy or as palliative treatment of advanced disease. In recent years the

IMRT technique has been used to allow a better tolerance and optimization of the irradiation volumes. In stages I and II is indicated extrapleural pneumonectomy plus hemithoracic radiotherapy (IMRT). In resectable stage III patients undergo extrapleural pneumonectomy and hemithoracic radiotherapy (IMRT), with or without chemotherapy. Unresectable stage III due to N+ perform initial induction chemotherapy. If there is good response, patients are proposed for extrapleural pneumonectomy, hemithoracic radiotherapy (IMRT) and chemotherapy. In case of poor response patients are proposed for palliative chemotherapy with or without palliative radiotherapy, such as all patients in stage IV.
Conclusion: Only 5 to 10% of patients are candidates for radical surgery as first-line treatment. The remaining patients are candidates for less aggressive surgical procedures, associated with radiotherapy and chemotherapy complementary protocols. Depending on the stage and the general condition of patients, many candidates are only indicated for palliative chemotherapy or supportive measures.

BLADDER CANCER

P55. A NEW POSSIBLE APPROACH FOR THERAPY AND FOLLOW UP OF BLADDER CANCER

S. Ferreira, A.M. Abrantes, A. Brito, M. Laranjo, L. Metello, J. Zeevart, W. Louw, I. Dormehl, M.F. Botelho

Biophysics Unit, Institute for Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra, Coimbra; School of Sciences, University of Minho, Braga; Nuclear Medicine Course, High Institute of Allied Health Technologies of Porto's Polytechnic Institute, Porto; Centre of Investigation on Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra; Radiochemistry Department, NECSA, Pretoria, South Africa; Department of Internal Medicine, University of Pretoria, Pretoria.

Introduction: The polymer PEI-MP (polyethyleneimine, functionalised with methylphosphonate groups) that might be labelled with ^{188}Re and ^{99m}Tc, have a strong potential for metabolic radiotherapy and diagnosis, respectively. The aim of this study was to evaluate the efficacy of ^{188}Re-PEI-MP as therapeutic agent for bladder carcinoma and ^{99m}Tc-PEI-MP for its follow up.
Methods: Cytotoxicity of PEI-MP was investigated in bladder carcinoma cell line (CRL-1472) using the MTT test for different concentrations of PEI-MP (1 μM to 1000 μM) and incubation times (24h, 48h, 72h and 96h), and flow cytometry for a concentration of 1000 μM of PEI-MP (24h). Radiochemical purity of ^{188}Re-PEI-MP and ^{99m}Tc-PEI-MP was achieved using ascending microchromatography. Cellular uptake studies were performed using the complexes ^{188}Re-PEI-MP, ^{99m}Tc-PEI-MP, $Na^{188}ReO_4$ and $Na^{99m}TcO_4$. Cell samples were collected during four hours, centrifuged to separate supernatant and pellet. Subsequently, the radioactivity of each portion was counted to determine percentage of uptake. The *in vivo* studies were performed using eight groups of Balb/c nu/nu mice: four normal groups injected with $Na^{188}ReO_4$, ^{188}Re-PEI-MP, $Na^{99m}TcO_4$ and ^{99m}Tc-PEI-MP and four with bladder carcinoma xenotransplants injected with the same complexes. When tumour reached the appropriate volume, radiopharmaceuticals were administered by an intravenous injection in the tail vein (22-37 MBq), with the animal anesthetized and previously placed on the gamma camera detector. After injection of the radiopharmaceuticals, were acquired dynamic and static images for 2 and 4 hours. For biodistribution proposes, mice were euthanized 2 and 4 hours after injection and organ samples where weighted and counted in a well-counter to obtain percentage injected activity per gram of organ (%ID/g).
Results: The MTT assay and flow cytometry tests showed that PEI-MP is not cytotoxic. The radiochemical purity of ^{188}Re-PEI-MP and ^{99m}Tc-PEI-MP was ≥ 85%. The uptake studies demonstrated that the uptake was higher for ^{188}Re-PEI-MP and ^{99m}Tc-PEI-MP in relation to their controls, and higher for ^{188}Re-PEI-MP e relation to ^{99m}Tc-PEI-MP. Biodistribution results, with $Na^{188}ReO_4$ and $Na^{99m}TcO_4$, showed a higher uptake by the thyroid, bladder and stomach, following a normal biodistribution. The biodistribution with ^{188}Re-PEI-MP and ^{99m}Tc-PEI-MP showed that the excretion of these complexes occurs primarily through the renal system, with a small fraction being eliminated by the hepatobilliary system. Tumour/muscle ratio for ^{188}Re-PEI-MP was greater than 1.5.
Conclusion: Considering the results, ^{188}Re-PEI-MP seems to be promising in the treatment of bladder cancer. Following the same biodistribution as ^{188}Re-PEI-MP, ^{99m}Tc-PEI-MP seems to be optimal for diagnosis and follow up of therapy.

P56. NATURAL KILLER ADOPTIVE CELL TRANSFER TARGETING BLADDER CANCER STEM CELLS: PRELIMINARY RESULTS FOR FINE TUNNING STRATEGY

M. Ferreira-Teixeira, P. Rodrigues-Santos, B. Parada, F. Reis, C.M. Gomes

Laboratory of Pharmacology and Experimental Therapeutics, Institute of Biomedical Research in Light and Image (IBILI), Faculty of Medicine, University of Coimbra, Coimbra; Institute of Immunology, Faculty of Medicine, University of Coimbra, Coimbra; Immunology and Oncology Laboratory, Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra; Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra; Urology and Renal Transplantation Department, Centro Hospitalar Universitário de Coimbra, Coimbra.

Introduction: 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). Recently, it was found that less undifferentiated cancer cells are more susceptible to NK-mediated cytotoxicity than their differentiated counterparts, suggesting that CSC might be a suitable target for NK-cell based therapies.
Objectives: The aim of this work is to explore the role of CSC in the susceptibility of BC cell lines to NK cell mediated-based immunotherapy.
Methods: Two human BC cell lines (HT-1376 and UM-UC3) were assayed for their susceptibility to lysis induced by NK cells, previously isolated from healthy donors, using the CD107a-based degranulation assay. IFN-γ production by resting and activated NK cells was detected by intracellular staining in the presence of both tumoral cell lines. The presence of putative CSC was analyzed using the sphere-forming assay in serum-free medium and non-adherent conditions. Cells sensitivity to cisplatin (CIS) and methotrexate (MTX) was determined using the MTT-colorimetric assay.
Results: A subset of CSC was identified in the HT-1376 cell line that grew as spherical colonies in contrast to the UM-UC3 cell line that only formed cell aggregates. 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%). Upregulation 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%), although not significant ($p > 0.05$). Moreover, after co-stimulation of NK cells with IL-2 and IL-15, the IFN-γ production increases 4-fold in the presence of both

tumoral cell lines, as compared with unstimulated NK cells ($p < 0.05$). MTT results showed that sphere-forming HT-1376 cells are more resistant to CIS and MTX than the UM-UC3 cells. The drugs concentration required to inhibit cell viability in 50% (IC_{50}) for HT-1376 cells was of 7.45 ± 1.20 μM for CIS and of 0.18 ± 0.03 μM for MTX, significantly higher ($p < 0.05$) than that observed in the UM-UC3 cell line (CIS: IC_{50} = 3.98 ± 0.70 μM; MTX: IC_{50} = 0.04 ± 0.01 μM).

Conclusion: The sphere-forming HT-1376 cells are more chemoresistant than the UM-UC3 cells, which is probably related with the presence of CSC. Both BC cell lines are equally susceptible to NK cell-mediated cytotoxicity, independently of the presence of CSC. Activation with IL-2 and IL-15 potentiates the effector function of NK cells as assessed by IFN-γ release. NK-cell based immunotherapy might be an alternative approach to eliminate drug-resistant stem cells and prevent tumor recurrence.

P57. EVALUATION OF CISPLATIN-INDUCED EFFECTS ON FOUR BLADDER CANCER CELL LINES

M. Filipe, J. Ferreira, I. Gaivão, M.R. Pinto-Leite

Master degree students of Biotechnology for the Health Sciences, UTAD; CECAV - University of Trás-os-Montes and Alto Douro (UTAD); Cytogenetics Laboratory, Central Hospital of Trás-os-Montes and Alto Douro.

Introduction: Cisplatin, a platinum compound, is one of the most used chemotherapeutic agents in the treatment of several malignancies. Its primary target is DNA, with which established crosslinks. The genotoxic effects of cisplatin has already been associated with a reduction of cell proliferation. The argyrophilic NOR-associated proteins (AgNOR) reflect the proliferative activity of cells by means of light microscopy and silver-stain methods. The aim of this study was to evaluate the effect of cisplatin in four bladder cancer cell lines (5637, MCR, T24 and HT1376) by conventional cytogenetics and the morphometric analysis of AgNOR proteins.

Methods: The cell lines were incubated in 96-well culture plates with cisplatin (2.5 μg/mL and 1 μg/mL) for 72 h at 37 ºC (plus a 5-day recovery period with two medium changes), and non-drug treated controls were processed alongside the drug treated samples. Conventional cytogenetics analysis was performed with the protocols already established in the laboratory. AgNOR proteins were stained by the silver nitrate method and the analysis was assisted by image analysis software.

Results: Exposure to cisplatin gave origin to several chromosomal aberrations such as chromatid breaks, radials formations, pulverized chromosomes, condensed metaphases and minute and double minute chromosomes. The mean percentage of the AgNOR occupied area on the nuclei had an extremely significant reduction in 5637, MCR and T24 ($p < 0.001$), and a very significant reduction in HT1376 ($p < 0.01$).

Conclusion: Cisplatin is genotoxic, producing several macro lesions detectable by conventional cytogenetics on all four cell lines. AgNOR staining revealed a drastically reduced cell synthesis activity in all cell lines and, by extension, a decline in cell proliferation.

P58. ATORVASTATIN, OMEGA-3 FATTY ACIDS AND SALICYLIC ACID IN THE PREVENTION OF BLADDER CANCER IN A CARCINOGENESIS EXPERIMENTAL MODEL

B. Parada, F. Furriel, P. Nunes, L. Campos, A. Mota, F. Teixeira, A. Figueiredo, P. Santos, M. Teixeira, F. Reis

Laboratory of Pharmacology & Experimental Therapeutics, IBILI, Medicine Faculty, Coimbra University, Coimbra; Department of Urology & Renal Transplantation, CHUC, Coimbra; Center for Neuroscience and Cell Biology, Coimbra University, Coimbra.

Introduction and objectives: Bladder cancer is a prevalent tumour with high recurrence and progression rates despite adequate treatment. Pharmacological prevention, ideally with oral, safe and economically affordable agents could be a good option, especially in high-risk patients. Our purpose was to study the efficacy of Atorvastatin (Atorva) Omega-3 fatty acids (Ω2-3) and Salicylic Acid (SCA) in the prevention of bladder cancer in a carcinogenesis experimental model.

Methods: Wistar rats (10 weeks old) were divided in several study groups: (1) BBN 0.05% group (n = 25); (2) Atorva 3 mg/kg/day (n = 8); (3) Ω2-3 2000 mg/kg/day (n = 8); (4) SCA 250 mg/kg/day (n = 8); (5) Control groups (n = 4 for each control group). The study involved two phases: Phase 1 – from week 1 to week 8 bladder cancer was induced with BBN 0.05% in drinking water in groups 1 to 4. In groups 2 to 4, the drugs were given by oesophagus cannula, to study their tumoral prevention capacity; Phase 2 – from week 9 to week 20: phase for cancer development. At week 20 animals were sacrificed. Bladders were sent for H&E and immunohistochemical studies. Blood was collected for inflammatory, tumoral and redox markers. Gene expression profile was also performed. Biochemical parameters were done for safety studies. Animal laboratory best practices were followed.

Results: Main results are presented in the table. Rats treated with Atorva and SCA had a more favourable redox status and lower levels of pro-inflammatory and tumoral factors. Differences were also found in gene expression profile, with higher expression of pro-apoptotic genes in animals with no bladder cancer. Drugs did not induce any significant toxicity.

Conclusion: In our experimental study, atorvastatin and salicylic acid could reduce not only the incidence of bladder cancer but also the number and volume of tumors. This could be due a more favourable redox and inflammatory status. Omega-3 fatty acids did not show such good results despite a reduction in the number and volume of tumors. Pharmacological prevention is a possible option in bladder cancer and deserves further investigation.

Table P58

	BBN 0.05%	Atorva	Ω2-3	SCA	Controls
% Tumor	68% (17/25)	12.5% (1/8)	62.5% (5/8)	12.5% (1/8)	0%
Papillary Hyperplasia	100%	37.5%	87.5%	25%	0%
High-grade dysplasia	96%	12.5%	62.5%	12.5%	0%
Cis	52%	0%	0%	12.5%	0%
Tumors per rat	1 ± 0.82	0.25 ± 0.71	0.75 ± 0.71	0.13 ± 0.35	0
Tumor volume (mm^3)	66.97 ± 154.72	0.65 ± 1.84	0.72	0.13 ± 0.37	0

P59. GALACTO-CONJUGATED PORPHYRIN: THE EXPRESSION OF GALECTIN-1 AND ABCG2 TRANSPORTER AFFECTS THE PHOTOTOXIC RESPONSE OF BLADDER CANCER CELLS TO PHOTODYNAMIC THERAPY

P.M.R. Pereira, S. Silva, C.M.F. Gomes, H. Girão, J.A.S. Cavaleiro, C.A.F. Ribeiro, J.P.C. Tomé, R. Fernandes

IBILI, Pharmacology & Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Department of Chemistry & QOPNA, University of Aveiro, Aveiro, and Center of Investigation in Environment, Genetics and Oncobiology, Coimbra.

Introduction: Photodynamic therapy (PDT) combines a photosensitizer (PS), light and molecular oxygen to generate intracellular reactive oxygen species (ROS) which will destroy cancer cells. Our research is focused on the design and evaluation of PSs for enhanced PDT, by the addition of galactose units in the photosensitive molecule. The aim of this work was to evaluate the putative beneficial effects of the porphyrin $PorGal_8$ decorated with galactose units against bladder cancer cells (UM-UC-3 and HT-1376 cell lines).
Results: $PorGal_8$ uptake profile as a function of incubation time was higher in UM-UC-3 than in HT-1376 cells. $PorGal_8$ was non-toxic until light activation and after PDT it was more photo-active against UM-UC-3 cells. Galectin-1 protein levels were higher in UM-UC-3 than in HT-1376 cells. Using the sphere formation assay, the presence of cancer stem cells (CSCs) expressing ABCG2 (multi-drug resistance pump) protein in HT-1376 cells was detected. The inhibition of galectin binding sites, by pre-incubating cells with galactose, decreased the uptake and photo-toxicity of $PorGal_8$ in both bladder cancer cells. Photo-toxicity induced after $PorGal_8$-PDT was correlated with increased formation of intracellular ROS and visible effects on actin microfilaments. For HT-1376 cells, the percentage of ROS was low and the changes in actin organization were transient.
Conclusion: The increased uptake and phototoxicity of $PorGal_8$ in UM-UC-3 cells can be somehow related to the higher protein levels of galectin-1 in these cells, which may serve as docking sites for $PorGal_8$ on the cell membrane and probably, facilitating entry of $PorGal_8$. The transient changes in the organization of actin microfilaments of HT-1376 cells after $PorGal_8$-PDT, seem to indicate that F-actin may be involved in the mechanism of resistance in these cells to PDT. In addition, it is also possible that CSCs present in HT-1376 cell line offer some resistance after $PorGal_8$-PDT.
This work was supported by FCT (PEst-C/SAU/UI3282/2011-COMPETE) and the project PTDC/CTM/101538/2008, and ACIMAGO (Ref. 12/12).

P60. COMPARISON OF URINARY CYTOLOGY, NMP22 AND UROVYSION® IN THE DIAGNOSIS AND PROGNOSTIC OF BLADDER CANCER

P. Dinis, B. Parada, L. Campos, F. Reis, P. Nunes, A. Figueiredo, F. Teixeira, A. Mota, J. Barbosa Melo, I.M. Carreira

Department of Urology and Renal Transplantation, Coimbra University Hospital, Coimbra; IBILI: Ocular and Brain Pharmacology and Experimental Therapeutics Unit; Cytogenetics and Genomics Laboratory: Faculty of Medicine, University of Coimbra, Coimbra.

Introduction: The purpose of this work is to compare the fluorescence in situ hybridization (FISH) probe set Urovysion®, consisting of probes for chromosomes 3, 7, and 17 and for the 9p21 band, with conventional cytology and the urinary marker Nuclear Matrix Protein 22 (NMP22) in the diagnosis, prediction of aggressiveness and recurrence in patients with bladder cancer.
Methods: Prospective study performed in 55 patients with a bladder tumour diagnosed in cystoscopies performed at our Department, between October 2008 and April 2010. Before transurethral resection, urine samples were collected for FISH (Urovysion® assay), NMP22 and conventional cytology testing. These urinary markers were evaluated for overall sensitivity and results were correlated with histopathological stage, grade and the presence of Carcinoma in situ (Cis). We also studied the prognostic value of urinary markers in predicting tumour aggressivity and recurrence. For statistical analysis we used SPSS for Windows version 16.0.
Results: All patients had biopsy-proven urothelial cell carcinoma. From the 55 tumours, 28% were muscle invasive, 70% high grade and Cis was present in 34%. Overall sensitivity was best for Urovysion® (66.7%) in comparison to NMP22 (57.5%) or cytology (52.8%). A combination of the three markers (75.5%) or Urovysion® + NMP22 (69.4%), yield better results than Urovysion® alone but Urovysion® + cytology or NMP22 + cytology did not improve the results. When correlating urinary markers with histopathological data, Urovysion® showed to have the best diagnostic accuracy (table). Using the urinary markers to predict the malignant potential of the bladder tumour, Urovysion® positivity showed statistically significant association with muscle invasion (p 0.043), high-grade cancer (p 0.000) and the presence of Cis (p 0.023). It was also the only urinary marker that could predict tumour recurrence (p 0.030). NMP22 positivity was only related to the presence of Cis (p 0.018).

Table P60. Detection rate according to tumoral characteristics

	Urovysion®	NMP22	Cytology
Stage			
Non-muscle invasive	56%	46.4%	44.8%
Muscle invasive	100%	80.0%	83.3%
Grade			
Low-Grade	25%	38.5%	33.3%
High-Grade	89.5%	64.0%	60.9%
Cis			
Absent	50%	42.3%	41.7%
Present	90.9%	83.3%	72.7%

Conclusion: In our study, Urovysion® showed to have the best diagnostic accuracy and the only marker that could predict a more aggressive behavior and tumour recurrence, when compared to conventional cytology and NMP22.

P61. BLADDER CANCER – COMPLICATIONS AFTER CYSTECTOMY

G. Gomes, P. Nunes, B. Parada, R. Patrão, C. Bastos, A .Roseiro, A. Mota

Serviço de Urologia e Transplantação, Centro Hospitalar e Universitário de Coimbra, Coimbra.

Introduction and objectives: Radical cystectomy is a major surgery associated with high morbidity and mortality. The identification of risk factors could improve the outcomes. We evaluated the results and complications using a standardized method, and factors associated to surgery due to complications.
Methods: Retrospective review of clinical files of 94 patients submitted to cystectomy from 03.01.2006 through 15.12.2010. Minimal follow-up was 90 days. Complications during hospital stay were graded according to a 5-grade modification of the Clavien system. The statistical analysis was performed with the assistance of software PASW 18.0.
Results: Mean patient's age were 66.7 ± 11.37 years. Seventy five were male (2.53:1.0 female). Mean time on waiting list for

surgery were 34.1 ± 27.4 days, median of 23.5. The most common urinary diversion was ileal conduit in 55.3% of patients, followed by orthotopic neobladder in 24.3%, cutaneous ureterostomy in 17.0% and 3.2% without any diversion due to anuria secondary to end stage renal disease. Mean surgery time was 4h21m, higher when a confection of neobladder was performed (4h39m; p = 0.002). Majority of patients had stage pT ≥ 3a, with 53.2%, 70.0% had negative node disease (pN-). In those with pT≥3a, 47.8% had pN+ vs 11.4% in pT < 3a (p < 0.000). Fifty one patients (54.3%) had postsurgical complications during hospital stay: 6 (6.4%) had grade I, 24 (25.5%) grade II, 0 (0.0%) grade IIIa, 12 (12.8%) grade IIIb, 1 (1.1%) grade IV and 8 (8.5%) grade V. Hospital stay was longer in those with complications (28.0 days vs 12.6 ; p < 0.000). Within the follow-up period, 20 (16.0%) patients were operated due to complications. On Multivariate analysis (age, gender, time on waiting list, surgery duration, urinary diversion, pT, pN), pN was independently associated with surgery for complications (OR: 4.207; p = 0.023).
Conclusion: Our series had a high number of patients with non-organ confined disease (pT ≥ 3a). Elevated number of complications, leading to a longer hospital stay. The presence of lymph node disease was associated with the need for surgery because of complications.

RENAL CANCER

P62. COMPARISON OF RESULTS OF LAPAROSCOPIC PARTIAL NEPHRECTOMY RENAL IN LESIONS MINOR AND MAJOR 4CM

P. Dinis, S. Bollini, P. Nunes, A. Figueiredo, P. Moreira, B. Parada, A. Mota

Department of Urology and Renal Transplantation of CHUC, Coimbra.

Introduction and objectives: Surgical treatment of renal lesions has evolved substantially in recent years with the attempt to preserve renal function, maintenance of oncological outcomes and decreased morbidity in relation to radical nephrectomy. When technically feasible laparoscopic partial nephrectomy (LPN) is an increasingly used option. The authors present a study comparing this surgery in lesions smaller than 4 cm with ≥ 4 cm.
Methods: We reviewed the medical records and imaging of 109 patients underwent LPN between November 2005 and February 2011. We assessed the main data for the patient, tumor, surgery and evolution. Of the 109 patients, 66 (60.6%) were men and 43 (39.4%) were women. The mean age was 61.21 years (21-88 years). The lesions ≥ 4 cm, representing 26 cases (23.9%), while < 4 cm in 83 cases (76.1%).
Results: Malignant lesions in 76 cases (69.7%) and benign in 33 cases (30.2%). Mean follow-up was 27.6 months (1-67 months). The mean surgical time in lesions ≥ 4 cm, was 125, 6 min (40-180 min) and 98.8 min (40-150 min) in lesions < 4 cm (p 0.0001). The average bleeding lesions ≥ 4 cm, was 252.0 ml (50-800 ml) and 191.7 ml (0-1000 ml) in lesions < 4 cm (p 0.002). The average number of ports used in lesions ≥ 4 cm was 3.32 ports (3-5 ports) and at < 4 cm 3.15 ports (1-4 ports) (p 0.23). The excretory was opened in 10 cases (38.5%) in lesions ≥ 4 cm and 12 cases (14.4%) of lesions < 4 cm (p 0.01). The clamp was performed in 22 cases (84.6%) lesions ≥ 4 cm and in 62 cases (75.6%) of lesions < 4 cm (p 0.25). In the case of clamping the average time was 24, 5 min (17-30) in lesions ≥ 4 cm and 19.8 min (11-29 min) in lesions < 4 cm (p 0.0001). The complications (urinary fistula, arteriovenous fistula, conversion, haemoperitoneum, ATN and pneumoperitoneum) occurred in 7 cases (26.9%) lesions and lesions ≥ 4 cm in 5 cases (6.0%) of lesions < 4 cm (p 0.007). The difference between pre and post operative creatinine was 0.1 mg/dL in lesions ≥ 4 cm and 0.02 mg/dL in lesions < 4cm (p 0.01). The average hospital stay was 4.19 days (2-13 days) in lesions ≥ 4 cm and 3.12 days (1-9 days) in lesions < 4 cm (p 0.007). No cases of local recurrence or systemic metastasis.
Conclusion: The LPN in lesions ≥ 4 cm, has cancer control, similar to lesions < 4 cm, but has a higher morbidity, higher incidence of complications and days of hospitalization. The surgery is more demanding, more bleeding, surgical time, duration of vascular clamping and number of ports.

RADIATION EFFECTS

P63. ANTALGYC RADIOTHERAPY A CLINICAL CASE

D. Roda, D. Fardilha, R. Marques, T. Serra, F. Costa, G. Melo

Serviço de Radioterapia, IPOCFG, EPE, Coimbra.

Introduction: Pain control in patients with cancer represents a significant aspect of radiation therapy practice. Radiation therapy is one of the most effective, and often the only, therapeutic option to relieve pain caused by nerve compression or infiltration by malignant tumor. Chordomas are rare tumors (1-4%), malignant midline, which develop from embryonic remnants of the notochord present in the axial skeleton namely the sacrum, mobile column and skull base. Although histologically be considered low grade, with a pattern of slow growth, are locally aggressive and invasive behavior, are often recurrent, with metastatic potential. When located in the Sacrum they manifest clinically by pain associated with radiculopathy, and at a more advanced stage with bladder and intestinal neurological dysfunction. The treatment of choice is surgical removal of the lesion. As this is rarely feasible, the External Radiotherapy (RTX) acquires an important role hampered however by the radioresistance that these lesions usually present themselves.
Methods: The authors describe the case of a female patient, 76 years old, diagnosed with sacral chordoma with intense loin-sacred pain and lower limb functional limitation, pharmacologically uncontrolled. The lesion was deemed surgically unresectable and the patient has undergone RTX. The total dose administered was 60.0 Gy/30 fr/6 weeks, performed initially 40.0 Gy/20 fr, followed by 20 Gy/10 fr increment after clinical and imaging evaluation.
Results: The patient fulfilled the proposed Radiotherapy scheme without complications. Showed substantial clinical improvement regarding pain control, having regained full mobility in the lower limbs. However, there was no change in tumor volume imaging, when evaluating at 20fr, which confirms the highly radioresistant lesion.
Conclusion: Radiation therapy is a useful treatment modality in this type of tumor, despite its radioresistance, provides some degree of local control, and allows fulfilling the main objective of pain control and functional restitution of the lower limbs.

P64. HYPERBARIC OXYGEN FOR DELAYED RADIATION INJURIES

D. Roda, C. Fardilha, R. Marques, T. Serra, G. Melo

Serviço de Radioterapia, IPOCFG, EPE, Coimbra.

Introduction: One half of all cancer patients will receive radiation therapy at some point during the course of disease, about 50% of patient will be long-term survivors. Some will experience late radiation tissue injury (LRTI) developing months or years later. Hyperbaric oxygen therapy (HBOT) has been suggested as a treatment for LRTI based upon the ability to improve the blood supply to these tissues.

Methods: Randomised controlled trials comparing the effect of HBOT versus no HBOT on LRTI prevention or healing, obtained using searches of the Cochrane Central Register of Controlled Trials (The Cochrane Library 2012).
Results: There was some evidence that HBOT is more likely to achieve mucosal coverage with osteoradionecrosis. From single studies there was a significantly increased chance of improvement or cure following HBOT for radiation proctitis, and following both surgical flaps and hemimandibulectomy. There was also a significantly improved probability of healing irradiated tooth sockets following dental extraction. Trials suggest that for people with LRTI affecting tissues of the head, neck, anus and rectum, HBOT is associated with improved outcome. There was no evidence of benefit in clinical outcomes with established radiation injury to neural tissue, and no data reported on the use of HBOT to treat other manifestations of LRTI.
Conclusion: Hyperbaric oxygen has shown consistent benefit in treating some patients with delayed radiation injury. Further research is required to establish the optimum patient selection and timing of any therapy. An economic evaluation should be undertaken.

MISCELLANEOUS

P65. CELLULAR AND MOLECULAR MECHANISMS OF MECHANOTRANSDUCTION INVOLVED IN METASTASIS - AN *IN VITRO* STUDY IN HEPATOCARCINOMA AND BREAST CANCER CELL LINES

J. Paulo, D. Matias, J. Balça-Silva, J. Simões-Correia, H. Girão, A.B. Sarmento-Ribeiro, J.M. Nascimento Costa

IPO, Coimbra; Faculty of Medicine, University of Coimbra (FMUC), Coimbra; Centre of Ophthalmology, IBILI, FMUC, Coimbra; Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), FMUC, Coimbra; University Hospital of Coimbra (HUC/CHUC), Coimbra.

Introduction: Invasiveness of tumors requires several distinct cellular functions including lost of cellular adhesion, cell detachment, altered motility and extracellular matrix interaction. This interaction between cells and its surrounding tissues involves mechanical stimuli that are transmitted into cells and translated into a biochemical response-mechanotransduction. The increased mechanical forces made by a stiff microenvironment would contribute to the aggressiveness of those tumors.
Objectives: The main objectives of this work were to study the effects of increased pressure on hepatocarcinoma and breast cancer cells proliferation, migration, adhesion, cytoskeleton organization and invasiveness.
Methods: To attain these objectives, a breast cancer and hepatocellular carcinoma cells lines, the MCF-7 and HUH-7 cells, respectively, were submitted to a constant increase of atmospheric pressure, 40 mmHg and 60 mmHg, in an airtight box during 4 hours. We analyzed: 1. Cell viability followed by propidium iodide cell cycle evaluation assay; 2. Cell migration by the scratch assay; 3. the expression of integrins by western blot using specific antibodies and 4. the actin cytoskeleton by fluorescent microscopy . Invasiveness was accessed using an invasion kit with a polycarbonate basement membrane.
Results: Increasing pressure caused an increased in the viability (despite not statistically significant) at 4h after the mechanical stimulation, and that was sustained for 24h. Those results were complemented with cell cycle analysis that revealed an increment in the cells in S-phase confirming the proliferative effect. Moreover, we observed a pressure-dependent single-cell detachment pattern on both cell lines when compared to controls. This migratory pattern was further explored by studying the cell-cell and cell-substrate adhesions molecules (E-cadherin and β-catenin, and β1integrin, respectively) which revealed decreased. This could be integrated in an amoeboid migration variant of the single-cell migration pattern. Besides that, cells submitted to pressure presented an increase in stress fibers, a cortical actin pattern, lamelipodia and filopodia. However, those effects were more intense in HUH-7 rather than in MCF-7 cells, suggesting a role of this mechanisms in the higher invasiveness phenotype.
Conclusion: In this work, we demonstrated that the mechanical stimuli have a critical role in migration and invasion, in the breast cancer and hepatocellular carcinoma cells. Our results open new perspectives on the role of mechanotransduction in cancer metastasis of. The exploration of the signaling pathways involved could represent future possible therapeutic targets.

P66. THE INFLUENCE OF SULFATHIAZOLE ON THE MACROALGAE *ULVA LACTUCA*

S. Leston, M. Nunes, I. Viegas, C. Nebot, A. Cepeda, M.A. Pardal, F. Ramos

CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra; CEF - Center for Pharmaceutical Studies, Health Sciences Campus, Pharmacy Faculty, University of Coimbra, Azinhaga de Santa Comba, Coimbra: CNC - Centre for Neurosciences and Cell Biology, Department of Life Sciences, University of Coimbra, Coimbra: Departamento de Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo.

The occurrence of pharmaceuticals in natural ecosystems has only recently been acknowledged as an important issue that must be addressed. The presence of these emerging contaminants is primarily a result of human activities from their administration in human and veterinary medicine for prophylaxis and therapeutic treatments and also to improve feed efficiency. Sulfonamides (SA) are a class of antibiotics routinely found in environmental matrices and therefore their role as contaminants should be investigated in non-target organisms. The present experimental work has evaluated the exposure of the chlorophycean *Ulva lactuca* L. to sulfathiazole (STZ), a SA drug commonly used in aquaculture, at two concentrations representing prophylactic (25 μg mL^{-1}) and therapeutic (50 μg mL^{-1}) administrations. Results showed that STZ presents high stability in seawater with only 18% degradation over the 5 days assay at both dosages tested. Also, macroalgae demonstrated an efficient uptake capacity with constant internal concentrations after 24h regardless of the external solutions and thus should be considered as a bioindicator species in risk assessment. As for the influence of STZ on growth, it induced a slight inhibition after 96h at both concentrations, which is likely related to mechanism of action of the drug disrupting the folate synthetic pathway.

P67. HERB-DRUG INTERACTIONS AS ONE OF THE POSSIBLE CAUSES OF CHEMOTHERAPY FAILURE

M.G. Campos, M.L. Costa, P. Carvalho, C. Batista, N. Jesus, S. Vitorino, T. Silva, A.P. Leite, C. Bento, R. Silva, I. Pazos, H. Gervásio

Observatory of Herb-Drug Interactions (OIPM) & Drug Discovery Group, Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Santa Comba, Coimbra; Serviço de Nutrição do Instituto Português de Oncologia de Coimbra Francisco Gentil, E.P.E. (IPOCFG, E.P.E.), Coimbra; Serviço de Oncologia Medica do Instituto Português de Oncologia de Coimbra Francisco Gentil, E.P.E. (IPOCFG, E.P.E.), Coimbra.

Introduction: The exponential increase of the number of people with cancer in the next 20 years can leads to a collapse of the National

Health System in Portugal. All the help is welcome to implement some sustainability. Data from INFARMED in 2011, related to 2010, indicates that cancer treatments are in the third position in Health costs with 21% (221,913,911€) for antineoplastics in Hospital care. It is crucial to establish the cause of the unsuccessful treatments, to make them more effective and to reduce the costs in health and economy. One unexplored cause is herb-drug interactions. These situations, especially in oncologic patients, have a high fee at human's life and consume resources that aren't certainly counted. If some cases could be scrutinized before the failure of treatment, using inexpensive screening methods would be helpful to reduce costs. The goal of our work is to understand herb-drug interactions resulting from concomitant oncologic regimens and the intake of herbs and herbal extracts used in self-medication by our patients. It is also intended to provide tables with these interactions to Clinicians in order to prevent that chemotherapy cycle fails due to the concomitant use of natural products during the treatment.
Methods: In this research we worked with patients signalized in IPOCGF, E.P.E (Projecto ICi-Plant: Interacções Citostático-Planta) where "natural products" and chemotherapy coexisted. For better understanding of these cases we will propose tables for herbal-drug interactions identification.
Results: Data from relevant Clinical cases studied in OIPM revealed that main herbs used by patients are Aloe, Angelica, Curcuma, Ginseng, Green tea, Milk thistle, Noni, "Pau d'arco", various mushrooms as Shytake and Maitake and food as concentrated fruit juices of Citrus and Beet, Anona, Mangosteen and Goji berries. The evaluation of potential herb – drug interaction is the first step based on theoretical data and presented as Tables involving also cytochrome P450 isoenzymes. For instance Asian Ginseng, Garlic, Goji berries, Goldenseal, Licorice, Mangosteen, Milk thistle, Saw palmetto, which are CYP 2C9 inhibitors, can increase bioavailability of anticancer drugs such as Carmustine, Idarubicin and Tamoxifen (CYP 2C9 substrates) exposing the patient to toxic side effects. However herbs as American Ginseng, Danshen and St. John's Wort induce CYP 2C9 isozoenzymes, reducing these anticancer drugs bioavailability which can result in a treatment failure.
Conclusion: These natural products are available everywhere, easy to take as self-medication, hidden from Clinicians knowledge. Highlighting these situations and increasing their awareness is crucial to help avoiding further chemotherapy failures.

P68. POTENTIAL PHARMACOKINETIC HERB-DRUG INTERACTIONS WITH R-CHOP CHEMOTHERAPY

M.L. Costa, M.G. Campos

Observatory of Herb - Drug Interactions (OIPM) & Drug Discovery Group, Center for Pharmaceutical Studies, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Santa Comba, Coimbra.

Introduction: CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone) has been used for the treatment of Non-Hodgkin Lymphoma for some decades. Since clinical trials showed higher overall survival when Rituximab was added to the CHOP protocol (as compared with standard CHOP alone), R-CHOP became one of the first-line treatments for Non-Hodgkin Lymphoma. Anticancer drugs as cyclophosphamide, doxorubicin and vincristine have narrow therapeutic ranges and a small variation from that range can jeopardize the treatment or increase the toxic levels to which the body is exposed. Both situations are unwanted, and in order to predict that risk is necessary to know and control factors which can cause them. Herbal medicines are highly consumed among cancer patients and their use is understood as safe and miraculous, and therefore herbs are not faced as dangerous when taken simultaneously with chemotherapy.
Objectives: The aim of this work is to identify potential herb-drug interactions with the R-CHOP regimen.
Methods: A literature survey was done using the following search engines: Elsevier-Science Direct, PubMed, SpringerLink, Web of Science (ISI), Web of Knowledge (WOK) and B-on. The search included the following keywords: *pharmacokinetics, CYP, herbs, herb-drug interaction*. Additional sources were obtained from manual searches of recent journal articles and textbooks. After doing a pharmacokinetic characterization of drugs and herbs, the data were crossed and potential herb-drug interactions were identified.
Results: Drugs which compose the R-CHOP regimen are mainly metabolized at CYP 2B6 and CYP 3A4. Thus, herbs that can inhibit or induce their hepatic metabolism can modify their bioavailability and outcomes. For instance, dashen, gingko, echinacea and St. John's wort has been described in the literature as inducers of CYP 3A4 decreasing the serum amount of drugs metabolized in that CYP enzyme. On the other hand, herbs that inhibit drugs metabolism, increase their plasmatic serum levels, what can be very dangerous when we are dealing with anticancer drugs. Herbs such as aloe, Asian ginseng, licorice, garlic, milk thistle, valerian or saw palmetto are commonly used by cancer patients, and have the ability to inhibit CYP3A4. Their potential to interact with cyclophosphamide, doxorubicin and vincristine is high.
Conclusion: Various herbs commonly used among cancer patients, can potentially change the bioavailability of drugs from R-CHOP. That can jeopardizes chemotherapy treatments inexplicably when health professionals are not aware of their consumption. Health professionals should be attentive to the use of herbal medicines, inquire and register that information in the patient's medical record.

P69. THE RISK OF HERB-DRUG INTERACTIONS IN THE PERIOPERATIVE PERIOD

M.L. Costa, M. Castel-Branco, A.A. Marques, M.G. Campos

Observatory of Herb-Drug Interactions & Drug Discovery Group of Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra; Observatory of Herb-Drug Interactions & Health Surveillance Group of Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra; Anesthesiology Department, Hospital and University Center of Coimbra, Coimbra.

Introduction: Herb-drug interactions that occur in the perioperative period may have a significant impact in the surgical procedure and recovery of a patient. Surgical emergencies do not allow detailed drug history, and probably do not include questions regarding herbal medicines. As a matter of fact, (scheduled) surgeries are an example where the medication reconciliation is very important to a successful procedure and recovery. The number of drugs used in perioperative period increases significantly the risk for drug-drug interactions and that risk is extended to herb-drug interactions. The aim of this work is to identify potential herb-drug interactions between drugs used in the intra-operative period and herbal medicines taken in the perioperative period.
Methods: A literature survey was done using the following search engines: Elsevier-Science Direct, PubMed, SpringerLink, Taylor & Francis, Web of Science (ISI), Web of Knowledge (WOK) and B-on. keywords: *pharmacokinetics, pharmacodynamic, CYP, herbs, medicinal herbs, herb-drug interaction*. After doing a pharmacokinetic and pharmacodynamic characterization of drugs and herbs, the data were crossed and potential herb-drug interactions were identified.
Results: It is relevant to safeguard that the data referred to herbs characterization is complex, incomplete and sometimes confusing or controversial. But the goal of this work is to alert to potential

herb-drug interactions, taking into account the current available data. When patients are taking herbs that can modulate the activity of CYP enzymes, inhibiting or inducing them, the metabolism of their substrates is altered. For instance, the consumption of aloe, goldenseal, vinca and other herbs can inhibit the expression of CYP 2D6 which can decrease the amount of tramadol converted to M1 (its active metabolite). Thus, tramadol's analgesic effect may be decreased because of this less or non-conversion. Anesthetic drugs as sevoflurane, propofol, halothane, levobupivacaine, bupivacaine, lidocaine are drugs that can increase the risk of bleeding. If taken concomitantly with herbs containing *coumarin-like* molecules (with similar effect as warfarin), herbs containing salycilates (with similar effect as aspirin), or herbs that inhibit platelet aggregation, the risk of bleeding intensifies.
Conclusion: The clinical outcome of such interactions is difficult to predict because the extracts of such herbs are not standardized, and generally is difficult to know what exactly their content is. Nevertheless, more research is vital to improve our knowledge in the field. Beyond that, the rapid growth in consumption of these products requires fast adaptation of health professionals to this scenario. It is imperative to prepare training programs in this field, and to learn from the information already published. It is also important to detect and notify all the situations suspected of herb-drug interaction, in order to validate (or not) theoretical information about potential interactions.

P70. ROLE OF DENTISTRY IN THE OSTEONECROSIS OF THE JAW ASSOCIATED WITH BISPHOSPHONATES

S. Paulo, A.M. Abrantes, M. Laranjo, L. Carvalho, A. Serra, M.F. Botelho, M.M. Ferreira

Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra; Department of Dentistry, Faculty of Medicine, University of Coimbra, Coimbra; CIMAGO, Faculty of Medicine, University of Coimbra, Coimbra; Institute of Pathological Anatomy, Faculty of Medicine, University of Coimbra, Coimbra ; Chemistry Department, Faculty of Science and Technology, University of Coimbra.

Introduction: Ostenonecrosis of the jaws (ONJ) is an adverse effect of bisphosphonates therapy, used for the treatment of bone metastasis, namely Zoledronate, the most potent and widely used nitrogen Biphosphonate in breast, prostate and lung cancers and in multiple myeloma.
Objectives: Once it is an emerging problem with few therapeutic options, our purpose is to study different materials that could be applied in the oral wounds, favoring it cicatrization.
Methods: In chemistry laboratory, it was studied the reaction of Zoledronate with different compounds, in order to find a compound that can efficiently bind Zoledronate. The cells in which this compound is going to be tested are primary cultures of gingival fibroblasts, cultivated following a specific protocol that included the collection of tissue from a oral procedure and it manipulation in laboratory, in order to obtain cell multiplication. In the animal model, there were used Wistar rats, and it was compared the cicatrization of oral wounds after an oral surgery in different groups: with and without Zoledronate weekly subcutaneous administration. Zoledronate was marked with Technetium^{99m} to monitor which were skeleton zones that capture more Zoledronate, highlighting where bone turnover was more active.
Results: Our results demonstrate that exist materials capable of Zoledronate's capture. In animal models we confirm that wound cicatrization was delayed in the group with Zoledronate administration. Our results suggest that the application of studied compound can decrease the toxicity of Biphosphonate and possibly help to reverse Biphosphonate related ONJ.
Conclusion: Once there in no known an effective resolution for ONM, patients with bisphosphonate treatment should have a preventive oral healthcare combined with nonsurgical dental procedures to reduce the need of tooth extractions because of extensive caries or periodontal conditions. There is a huge necessity of advances in investigation once this emerging problem is taking away patients quality of life.

P71. A MULTIFACTORIAL INTEGRATIVE MODEL TO PREDICT EXTERNAL APICAL ROOT RESORPTION ASSOCIATED WITH ORTHODONTIC TREATMENT

S. Alves, N. Lavado, L. Mesquita, P. Pedro, M. López, M. Abreu, H.C. Silva

Department of Orthodontics, Dentistry Area, Faculty of Medicine, University of Coimbra, Coimbra; Coimbra Institute of Engineering (ISEC); Medical Genetics Department, Faculty of Medicine, University of Coimbra, Coimbra; Institute of Mechanical Engineering, Faculty of Engineering, University of Porto, Porto.

Introduction: External apical root resorption (EARR) is the most common clinical sequela and undesirable side effect of orthodontics. It is a complex phenotype, being determined by poorly identified mechanical and patient intrinsic factors. Also, there is no standardized method for diagnosis. This work aimed to develop a reliable and practical method to measure orthodontic-induced EARR from panoramic radiographs and to evaluate the contribution of several clinical and genetic factors in order to construct a multifactorial integrative model to analyze the risk of developing this common orthodontic complication.
Methods: This retrospective study included 193 patients, treated with fixed appliances. Panoramic radiographs were used to measure root resorption, before and after treatment, in the maxillary incisors and canines. Ten clinical and treatment variables were evaluated for each patient. A design-to-purpose software was developed to allow image processing, introduction of a magnification correction factor and automatic computing of teeth root resorption percentage. For each patient, we considered the tooth with maximum % of root resorption (EARRmax). Polymorphisms of four candidate genes (rs1143634 from *IL-1B*, rs3102735 from *OPG*, rs1805034 from *RANK* and rs1718119 from *P2RX7*) were evaluated by RFLPs and TaqMan assay. A multiple linear regression model was used to analyzed the contribution of all variables to the occurrence of EARR.
Results: The analysis of intraobservational mean error for root resorption measurements confirmed the reliability of the method ($p > 0.05$ in Student's t test for paired samples). Hardy-Weinberg equilibrium was verified for all four polymorphisms. Clinical and genetic variables explained about 30% of the EARRmax variability (ANOVA: $F = 17.208$, $p = 0.000$; adjusted determination coefficient = 0.297, $n = 193$). The variables with more significant unique contribution to the model were: treatment duration ($p < 0.001$), Hyrax appliance ($p < 0.001$), premolar extractions ($p < 0.01$), gender ($p < 0.05$) and heterozygosity for *P2RX7* gene polymorphism ($p < 0.01$). Other variables, like age, fixed functional appliance, overjet, tongue thrust, anterior open bite, skeletal pattern and the other polymorphisms had minor contributions.
Conclusion: Software-assisted analysis of panoramic radiographs is a reliable method to evaluate orthodontic-induced EARR. This study highlights *P2RX7* gene as possible susceptibility factor. A more extensive genetic profile may improve this model.