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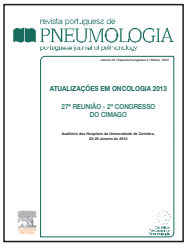

ORAL PRESENTATIONS

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The abstracts are the author's responsibility.

CANCER AND ENVIRONMENT

OP1. ASCORBIC ACID AND ITS CYTOTOXIC EFFECT IN MELANOMA: IN VITRO AND IN VIVO STUDIES

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Introduction: Malignant melanoma is a type of skin cancer that affects younger population. In the metastatic stage it is extremely difficult to treat and does not respond to current therapies. Ascorbic acid (AA) is the reduced form of vitamin C. As an antioxidant, the main role of vitamin C is to reduce oxidative stress. However, this nutrient may have a pro-oxidant activity, promoting the formation of reactive oxygen species (ROS) that can induce cancer cell death revealing a potential therapeutic of AA in cancer. The increased production of hydrogen peroxide, coupled with the breakdown of the activity of antioxidant enzymes and the presence of transition metals in cancer cells, may result in the selective cytotoxicity of vitamin C. The aim of this study is to evaluate, in vitro and in vivo, the cytotoxic effect of AA in a melanocytic melanoma cell line.
Methods: A-375 cells were incubated with different concentrations of AA (0,25-10 mM). The half maximal inhibitory concentration (IC50) was calculated after 24, 48, 72 and 96 hours by the sulphorhodamine B assay. In order to evaluate cell survival, clonogenic assays were performed. Flow cytometry was performed to determine cell viability and death, ROS production, alteration of mitochondrial membrane potential and cell cycle. In order to verify in vivo the evolution of tumor growth, Balb/c nu/nu xenografts were daily submitted to intraperitoneal therapy with AA.
Results: AA induces a decrease in cell proliferation and survival in a dose dependent manner ($r^2 > 0.97$), being the IC50 less than 1,6 mM. AA also induces a cytotoxic effect when cells are treated with 10 mM of AA, being with this dose also observed a decrease in intracellular peroxides and an increase in intracellular superoxide radical and cell cycle arrest. Our studies also show a decrease in the ratio aggregates/monomers in a dose dependent way. The in vivo studies suggest that AA administered daily at 150 mg/kg inhibits tumor growth.
Conclusion: AA induces a decrease in cell survival, proliferation and viability in A-375 cells, correlated with cell death by apoptosis and increased reactive oxygen species, results that are supported by the in vivo studies. These results suggest that AA may have a potential anti-cancer effect in melanoma cell lines.

OP2. JOINING PHOTODYNAMIC THERAPY AND ASCORBIC ACID AGAINST CANCER

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Introduction: Photodynamic therapy (PDT) relies on the use of a photosensitizing substance that undergoes activation by light with a specific wavelength. This therapeutic modality results in an

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increase of oxidative stress by ROS production that are responsible for irreversible damage in tumor tissues. Ascorbic acid (AA) is commonly known as an antioxidant agent. However, in pharmacologic concentrations, it can act as a prooxidant agent. Thus, this work aimed to evaluate a possible synergistic effect of the combination of AA with PDT regarding oxidative stress and cell death.
Methods: The evaluation of the photodynamic action of the sensitizer (PS) combined with AA was performed in a human cell line of colorectal adenocarcinoma (WiDr) and esophagus carcinoma (OE19). To ascertain the influence of AA in the photodynamic effect, assays to determine changes in cell proliferation, viability, ROS production, expression of antioxidant defenses, mitochondrial membrane potential, cell cycle and cell death pathways were held. It was also performed fluorescence microscopy studies to determine the intracellular location of the compounds, and finally, in vivo therapeutic outcome.
Results: The lysosomes and mitochondria appear to be the sites of subcellular location of the sensitizer regardless the presence of AA. In both cell lines, the combination of AA with PDT inhibited cell proliferation. Higher concentrations of PS resulted in the increase of cell death by late apoptosis/necrosis, mitochondrial membrane depolarization, ROS production, in which singlet oxygen had a prevailing role. In vivo studies resulted in the decrease of tumor volume regarding OE19 cell line.
Conclusion: The results obtained by the combination of AA with the photodynamic treatment in vivo, even though preliminary, show a decrease of tumor volume in the OE19 cell line. These results are in agreement with the in vitro studies, which indicate a synergistic effect of the combination of these two therapies.
Funding: Science and Technology Foundation SFRH/BD/44957/2008 grant to M. Laranjo.

GENETICS AND GENOMICS

OP3. INTERFERENCE OF HOST GENETIC PROFILE IN PREDICTING RESPONSE TO CHRONIC HCV INFECTION

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Introduction: In hepatitis C virus chronic infection, response to pegylated interferon α (PEG-INF) and ribavirin (RBV) combined therapy is successful in around 50-60% of patients. Recently, SNP rs12979860, located upstream of the IL28B gene, was identified as a genetic marker of response to PEG-IFN/RBV treatment. In ABCB11 gene, encoding the bile salt export pump protein (BSEP), the SNP rs2287622 (p.V444A, c.1331T > C) is now a well-established susceptibility factor for acquired cholestasis and recent evidence suggests that it also influences progression of viral hepatitis C. As new strategies with HCV direct-acting antiviral drugs are emerging, identifying predictive markers for individualized treatment will be crucial. We evaluated the correlation between these two polymorphisms and the response to INF-combined therapy.
Methods: 117 patients with chronic HCV infection were included in this study. Peripheral blood was collected for patient genomic DNA and viral RNA extraction. Genotyping of rs12979860 and V444A polymorphisms were determined by automated sequencing and RFLP assay, respectively. The viral load was assessed using the test COBAS® AmpliPrep/COBAS TaqMan® HCV® (Roche). The identification of hepatitis C virus genotype was performed with 2.0 VERSANT® HCV Genotype (LiPA) assay. Sustained virologic response (SVR), defined as undetectable levels of HCV RNA 24 weeks after completion of therapy, was considered the end point of treatment.
Results and discussion: For rs12979860, 12.8% of patients had the risk genotype, TT. Patients homozygous or heterozygous for the C allele had a significant increased probability of achieving SVR than patients homozygous for T allele ($p < 0.01$; OR = 6.1; IC95%: 1.88-19.79). As for V444A (T > C), almost 30% of patients were homozygous for C allele, associated with lower expression of BSEP, and only 15% were homozygous for T allele. No significant association was found with response to therapy, though patients with C allele achieved SVR less frequently than homozygous for T allele. Other markers of poor response were HVC genotype 1 or 4 ($p < 0.003$; OR-6.3; 95%CI-1.95 to 20.38) and absence of early viral response (EVR, reduction in serum HCV RNA levels by at least 2 log 10 IU/ml by week 12), with no patients who did not obtain an EVR at 12 weeks of treatment, achieving SVR. High risk genotypes 1 and 4 were identified in 67% of patients.
Conclusion: IL28B associated SNP, HCV genotype and EVR had a good correlation with the outcome of INF-combined therapy. Further studies with larger samples are needed to better evaluate the role of ABCB11 V444A polymorphism.

OP4. OLIGONUCLEOTIDE ARRAY-CGH AS A FIRST TIER TEST FOR THE DIAGNOSIS OF PATIENTS WITH INTELLECTUAL DISABILITY, MULTIPLE CONGENITAL ANOMALIES AND AUTISM SPECTRUM DISORDERS

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Introduction: Microarray-based comparative genomic hybridization (array-CGH) is nowadays the first genetic test offered to detect genomic imbalances in patients with intellectual disability with or without dysmorphisms, multiple congenital anomalies, learning difficulties and autism spectrum disorders. It allows the possibility to screen the whole genome at once and with high resolution, increasing the diagnostic yield and identifying Copy Number Variants (CNVs) sometimes challenging to interpret.
Methods: In a 20 month period, 500 patients were analysed with Agilent 180K whole genome oligonucleotide microarrays. The observed imbalances were classified in five main classes depending on the genetic content, overlapping with known microdeletion/duplication syndromes and existence of previous described CNVs in normal individuals.
Results: In 195 patients of the studied cohort, 269 imbalances were identified, 120 deletions, 147 duplications an even two tetrassomies, that belong to a class of pathogenic or potentially pathogenic imbalances. 62.5% of these imbalances had a genomic size between 1.985 Kb and 400 Kb, and 16% between 1-5 Mb, with the remaining distributed by different genomic ranges up to 53 Mb. Of the imbalances whose origin is known, 41.8% were maternal in origin, 35.2% paternal, 0.6% inherited from cousin progenitors, corresponding to one of the tetrassomies, and 22.4% de novo. In the studied cohort, 22 patients revealed to have known microdeletion or microduplication syndromes, eleven de novo, three paternal, one maternal and seven of unknown origin.
Discussion: We detected a total of 291 pathogenic or potentially pathogenic imbalances in 500 patients, with known microdeletion

or microduplication syndromes included. We concluded that inherited CNVs have a smaller genomic size than de novo CNVs. The great majority, almost 77%, of all the inherited CNVs identified were smaller than 500 Kb, while 35% of the de novo imbalances had a genomic size between 1-3 Mb.

GYNECOLOGIC ONCOLOGY

OP5. INTERFERENCE OF PROTEASOME INHIBITOR MG262 WITH WNT AND NF-$_\kappa$B PATHWAYS IN AN OVARIAN CANCER CELL-LINE

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Introduction: Proteasome inhibitors (PI) are recently introduced cancer target drugs with great efficacy in some hematologic malignancies. Nevertheless, for solid tumors, less convincing results have been reported. A better understanding of how PIs act in the signaling pathways in specific types of cancer is important to find predictive biomarkers to allow the selection of subpopulations of patients that will respond to these agents. The aim of this study was to analyze PIs mechanisms of action in ovarian cancer, focusing in two frequently involved signaling pathways, Wnt and NF-$_\kappa$B, for which antagonistic responses are expected.
Methods: TOV-112D, an ovarian cancer cell line with an activating β-catenin mutation and loss of P53 function, was exposed to different concentrations of PI MG262, for 24 and 48 hours. By flow cytometry we analyzed the type of cell death with Annexin V-APC/Propidium Iodide assay, the inhibition of the proteasome by the variation in ubiquitinated-protein levels using Anti-Ub P4D1-FITC and the level of NF$_\kappa$B activation using mouse anti-human NF-kB p65 (pS529)-PE mAb. To assess interference with Wnt pathway activation we quantify β-catenin nuclear translocation using indirect immunofluorescence and the expression of one of its target genes, CCND1, by qRT-PCR real time. Also by qRT-PCR real time, we evaluated interference with expression of hTERT, the catalytic subunity of telomerase, and SNAIL, a marker of epithelial-to-mesenchymal transition phenotype. Interference with invasive behaviour was evaluated with a wound-healing assay.
Results and Conclusion: After 48 hours of exposure to MG262, we observed a dose-dependent decrease in cell viability ($p < 0.05$), with a IC_{50} of 14 nM. The preferential type of cell death was necrosis. A significant inhibition of proteasome was accomplished with 10 nM ($p < 0.05$), without significant increase with exposure to higher concentrations. There was an increase in the levels of activated NF-$_\kappa$B with 5 and 10 nM ($p < 0.01$) of MG262, meaning that proteasome inhibition couldn't induce I_κB cytosolic accumulation or that I_κB was unable to complex and functionally inactivate NF-$_\kappa$B. On the other hand, although concentrations of 10 nM induced β-catenin nuclear translocation ($p < 0.01$), the expression of cyclin D1 gene (CCND1) decreased ($p < 0.05$). The mRNA expression of hTERT was also diminished with concentrations of 10 nM ($p < 0.05$). The decreased expression of CCND1, and hTERT, are in accordance with the effects of MG262 on cell viability and suggests that NF-$_\kappa$B and β-catenin couldn't succeed to induce expression of their target genes. MG262 didn't interfere with SNAIL expression, but it induced a reduced mobility in the wound-healing assay ($p < 0.001$). This decrease was achieved with 5 nM of MG262, and may be of interest in ovarian cancer, known by its characteristic superficial dissemination in peritoneum.
Conclusion: The interference of PI with NF-$_\kappa$B and β-catenin pathways is complex and apparently involves nuclear and not cytoplasmic molecular mechanisms, probably by impairing the transcription of target genes.
We acknowledge ACIMAGO for funding this work.

OP6. AGE-PERIOD-COHORT ANALYSIS OF BREAST CANCER MORTALITY IN PORTUGAL (1980-2009)

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Introduction: According to Statistics Portugal (INE), malignant neoplasms are the second leading cause of death in Portugal, claiming 24,982 deaths in 2008. Among them, colorectal cancer is the main cause of death, followed by lung, stomach and breast cancers, respectively. In this study, the results of an exploratory data analysis of breast cancer mortality rates in Portugal through an age-period-cohort (APC) analysis.
Objectives: The study present an APC of breast cancer mortality rates from a Lexis diagram between 1980 to 2009 in Portugal and according to NUTS II regions.
Methods: The database used came from official certified breast cancer deaths registries supplied by INE, and population estimates were calculated over the period 1980 to 2009 according to region, 5-year age and period intervals. Women at risk were calculated for the triangular subsets of the Lexis diagram and expressed in person-years. An APC was fit to the data using R software. For Portugal and each of the 7 regions analyzed, the best of the 5 models was chosen according to the deviance. In all hypothesis test a significance level of 0.05 was considered.
Results: There is a clear linear increase of the breast cancer rates with age in all regions. With the exception of Madeira, where only the period effect was statistically significant, an A-P-C model was the best fit for the data. In Lisbon the period effect was much stronger than the cohort effect, while in Alentejo and Center the opposite occurred. All regions showed a similar increased risk of breast cancer until the reference birth cohort (1930), with Center and Lisbon stabilizing after the 1940 birth cohort; Azores, Madeira and North showing no signs of a decreasing tendency; and Alentejo and Algarve suggesting a decreasing risk in women born after 1940 and 1955, respectively. The period effect shows a very similar behavior for all regions, with a decline in period-specific risk until around 1990 and increasing after that time.
Conclusion: Breast cancer mortality rates in Portugal and its regions show a global decrease after early 90´s. This can be explained by both opportunistic and organized screening activities. Regions were organized screening was earlier introduced and were population coverage was earlier reached, presented cohort effects stronger than period effects.

HEMATO-ONCOLOGY

OP7. OXIDATIVE STRESS VS GENE METHYLATION – A NEW RISK FACTOR IN MDS PATIENTS?

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Introduction: Myelodysplastic syndromes (MDS) are a heterogeneous stem cell disorders characterized by the underproduction of one or more blood cells types, due to hematopoiesis dysfunction, and higher risk of leukemic transformation. Oxidative stress (OS) contributes to cell damage, apoptosis and ineffective hematopoiesis and can be involved in MDS etiology and pathogenesis. Moreover, our previous studies show that p16 and p15 genes are inactivated by methylation in MDS patients. In this context, we investigate the relation between oxidative stress and p15 and p16 methylation status as risk factor and as prognostic marker in MDS patients.
Methods: We examined the expression levels of ROS, peroxide and superoxide anion, and the antioxidant defense GSH by flow cytometry using fluorescent probes, H2DCF-DA, DHE and mercury orange, respectively, in CD34 bone marrow cells collected at diagnosis from 24 patients with de novo MDS. DNA was isolated from bone marrow aspirates and modified by sodium bisulphite. The p15 and p16 promoter methylation profile were analyzed by MSP. The patient group median age was 77 years (33-84), gender M/F = 13/11, WHO subtypes: RCMD (n = 8), RA (n = 5), RAEB-1 (n = 2), RAEB-2 (n = 6), 5q- syndrome (n = 1), LMMC (n = 2) and IPSS: low (n = 6), intermediate-1 (n = 13) and intermediate-2 (n = 5).
Results: Our results show that CD34 MDS cells had higher expression of peroxide, although a significant lower expression of superoxide anion and GSH levels, compared to controls. However, ROS and GSH expression were subtype-dependent. Besides that, 50% of MDS patients have at least one methylated gene and 21% have both methylated genes. MDS patients who have at least one methylated gene show a significant increase in peroxide expression levels (565 ± 699 MFI) when compared with patients with unmethylated genes (230 ± 352 MFI), fact substantiated in patients with both methylated genes (878 ± 743 MFI). Besides that, superoxide and GSH levels were lower in patients with at least one (O_2^-, 180 ± 109 MFI; GSH, 165 ± 136 MFI) or both methylated gene (O_2^-, 183 ± 130 MFI; GSH, 156 ± 143 MFI), compared patients with unmethylated genes (O_2^-, 256 ± 136 MFI; GSH, 331 ± 569 MFI). Moreover, patients with high peroxide levels have an increase frequency of gene methylation (43%) and a lower survival, compared to patients with normal/low levels (12%).
Conclusion: This study suggests a correlation between oxidative stress, mainly peroxide levels, and gene methylation profile in MDS patients, since MDS patients with higher peroxide levels have higher frequency of gene methylation and lower survival.
This work was supported by CIMAGO (Project 22/09).

OP8. EPIGENETIC CHANGES IN MONOCLONAL GAMMOPATHIES – A POSSIBLE ROLE IN THE PROGRESSION OF MGUS TO MULTIPLE MYELOMA

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Introduction and objectives: DNA methylation profile was the earliest discovered epigenetic regulator and has been the focus of most investigations in cancer. Indeed aberrant methylation in promoter-associated CpG islands has been considered a crucial mechanism for tumor suppressor gene silencing in several malignancies. We have focused on DNA methylation changes in the pathogenesis of monoclonal gammopathies, namely its role in the progression from monoclonal gammopathies (MG) of uncertain significance (MGUS) to multiple myeloma (MM). In particularly, we aim to compare DNA methylation status of four genes (p15, p16, p53 and DAPK) in MGUS and in MM patients at diagnosis and to correlate MM DNA methylation changes with patient's features at diagnosis and with clinical prognosis.
Methods: We analysed bone marrow samples from 68 patients (pts) with MG at diagnosis, 42 MGUS (60% male, median age 72y, range 41-86) and 26 MM (42% male, median age 73y, range 39-86) and from 8 healthy donors, between June 2010 and October 2012. Samples were collected after informed consent according with the Declaration of Helsinki. Diagnosis of MM followed International Myeloma Working Group criteria (Br J Haematol, 2003). Methylation-specific polymerase chain reaction (MSPCR) for p15, p16, p53 and DAPK genes promoters methylation status was performed as previously described after DNA treatment with sodium bisulphite using a commercially available kit (EpiTect, Qiagen).
Results: Overall, 46% of pts with MG presented at least one hypermethylated gene (62% of MM pts and 36% of MGUS pts, $p < 0.05$). No aberrant methylation was detected in healthy donor's bone marrow. The frequency of hypermethylation for individual genes in MGUS and MM, respectively, was: p15, 14% and 15%; p16, 12% and 35% ($p < 0.05$); p53, 2% and 3%; DAPK, 17% and 39% ($p < 0.05$). The correlation between methylation status and clinical parameters was assessed. Pts with DAPK hypermethylation were more likely to have high serum β2-microglobulin levels ($p < 0.05$) and ISS 2 or 3 ($p < 0.05$). Aberrant p16 methylation was also associated with higher ISS 2 or 3 ($p < 0.05$) in MM pts and with DNA hyperdiploidy ($p < 0.05$) in pts with monoclonal gammopathies. We did not find any correlation between the methylation status of any gene and the presence of cytogenetic aberrations. Likewise, no correlations were observed between the methylation status of any gene and overall and progression free survival in MM pts.
Conclusion: We conclude that aberrant hypermethylation of tumor suppressor genes is a common event in pts with monoclonal gammopathies and there is a correlation between methylation patterns and several clinical features at diagnosis. Moreover, hypermethylation of p16 and DAPK genes might have a relevant role in the progression of MGUS to MM.
This work is supported by CIMAGO (project nº 23/09).

SOFT TISSUE AND BONE CANCER

OP9. TAC VERSUS PET IN THE DIAGNOSIS OF GANGLIONAR METASTASIS IN SOFT TISSUE SARCOMAS

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Objectives: Ganglionar metastases in soft tissue sarcomas are not common, but when present are associated to a worse prognosis. CT was used for the detection of ganglionar involvement is soft tissue sarcomas, but since 2005 PET-CT was included as a diagnostic tool for this specific diagnosis. The objective of the present study is to compare both imaging techniques in a group of patients also submitted to surgery of ganglionar resection based on the presumptive diagnosis obtained with the referred imaging studies.
Methods: Between 2005 and 2011, 52 soft tissue sarcoma patients were diagnosed also with metastatic ganglionar involvement, and submitted to surgery that included ganglionar resection, and their surgical specimens were studied for specific detection of ganglionar metastasis. 31 patients were female, 21 were male, with a median age of 53.6y. Locations of the primary lesions were axial in 24 patients and extremities in 28. The most frequent diagnosis was lipossarcoma, synovial cell sarcoma and soft tissue Ewing sarcoma. 40 patients (76.9%) were submitted in the same week to both exams, and 12 patients have the exams done with an interval superior to 3 weeks, and their results were excluded because of the time frame difference. A statistical analysis was performed using SPSS-19 version, and a Fisher exact test was use to compare results.
Results: The Fisher exact test was statistically significant when comparing the results of both imaging techniques ($p < 0.010$), not statistically significant ($p < 0.472$) when comparing the detected presence of ganglionar involvement in CT with the respective positivity in the surgical specimens, and statistically significant ($p < 0.006$) when comparing PET-CT results with the positivity in the surgical specimens. CT specificity was of 40% and sensitivity of 75% and a positive predictive value in 75% and a negative predictive value of 40%. PET-CT specificity was of 83.3% with a sensitivity of 86.7%, and a positive predictive value of 92.8% and a negative predictive value of 71.4%.
Conclusion: PET-CT has a significant positive predictive value, and higher specificity and sensibility in detection of ganglionar metastasis in soft tissue sarcomas.

OP10. TREATMENT OF ADENOCARCINOMAS WITH NEUROENDOCRINE DIFFERENTIATION WITH METABOLIC RADIOTHERAPY AND CHEMOTHERAPY

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Introduction: Pancreatic cancer, the fourth leading cause of cancer worldwide, is very resistant to surgery, chemotherapy and radiotherapy. MIA PaCa-2 is a human pancreatic duct adenocarcinoma cell line, a cancer that represents about 90% of all pancreatic tumours. This cell line expresses somatostatin 2 receptor. Metabolic radiotherapy is an optional treatment for pancreatic neuroendocrine tumors. The presence of adenocarcinomas with neuroendocrine differentiation raises the possibility of treatment with radiolabeled somatostatin analogs in association, or not, with chemotherapy agents. ^{99m}Tc-TOC and ^{177}Lu-DOTATATE are radiolabeled somatostatin analogs for diagnostic and therapeutic, respectively, of pathological lesions in which somatostatin receptors (specially subtype 2) are overexpressed. 5-FU (thymidylate synthase inhibitor) and everolimus (mTOR inhibitor) are cytostatic agents applied in treatment of pancreatic tumor.
Methods: To obtain uptake curves, MIA PaCa-2 cells were incubated with growing activities of ^{99m}Tc-TOC and ^{177}Lu-DOTATATE. Duplicated samples of pellet and supernatant were taken and the activity was measured in a well counter at 5, 30, 60, 90 and 120 minutes. The percentage of uptake was calculated by the ratio between the pellet and the pellet plus supernatant. IC_{50} of 5-FU and everolimus was achieved by the MTT assay. The type of cellular death was analyzed by flow cytometry, as well as the cell cycle.
Results: As the activity of ^{99m}Tc-TOC increases, the uptake of this radiopharmaceutical also increases. Related to ^{177}Lu-DOTATATE it appears that as the activity increases, uptake of this radiopharmaceutical is not enhanced. Cellular uptake of ^{99m}Tc-TOC is about four times higher than the uptake obtained with ^{177}Lu-DOTATATE. It was not possible to calculate the IC_{50} for 5-FU at 24 and 48 hours, but at 72 hours it was 49.83μM ($r^2 = 0.93$) and at 96h was 8.32μM ($r^2 = 0.93$). Everolimus IC_{50} were: 24h = 27,88μM (r^2 = 0,92); 48h = 20,09μM ($r^2 = 0,94$); 72h = 26,71 μM ($r^2 = 0,91$); 96h = 23,80μM ($r^2 = 0,90$). Celular death occurred in a dose-dependent manner and mainly by apoptosis. Drugs lead to an arrest of cell cycle at G_0/G_1 phase.
Conclusion: Although with different uptake rates, our results show that both radiolabeled somatostatin analogues could be successful applied in pancreatic cancer. Our results demonstrate that both radiopharmaceuticals probably don´t have equal affinity for SSTR2, being higher the affinity of ^{99m}Tc-TOC. 5-FU and everolimus inhibit cell proliferation, causing apoptosis and arrest of cell cycle. Our results should also be evaluated in the future in animal and in clinical studies.

CNS CANCER

OP11. HEMANGIOPERICYTOMA OF THE CENTRAL NERVOUS SYSTEM: A CLINICAL CASE

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Introduction: Hemangiopericytoma (HPC) is a malignant tumor arising from Zimmermann's pericytes around capillaries and postcapillary venules, more commonly located in the skin and musculoskeletal system. Central nervous system (CNS)-HPCs constitute less than 1% of all intracranial primary tumors. They can be confounded as meningiomas in imaging-scans; however, HPCs tend to present in younger patients and are more commonly seen in men. Radical surgery is the standard treatment, associated with adjuvant radiotherapy (RT) to the tumor bed. Although high, local recurrence in patients receiving doses greater than 51 Gy is lower and RT is strongly recommended, independently of the completeness of resection. Unlike other CNS primary tumors, CNS-HPCs metastasize to extra-CNS sites years after initial diagnosis. The aim of this work is to present a CNS-HPC clinical case, treated with surgery followed by RT, emphasizing the relevance of RT in its management.

Methods: A clinical case of CNS-HPC is described. Computed tridimensional RT planning is illustrated, considering tumor location and organs at risk in the surrounding area of the irradiation field. Treatment outcome and follow-up (FU) are presented.
Results: The patient was a 75-year-old male with history of cranial meningioma submitted to surgery who presented with dysphasia, loss of balance and periods of confusion 6 years after. Magnetic resonance imaging (MRI) revealed an expansive lesion located to the tentorium cerebelli, with important mass effect over the adjacent cerebrum, compatible with meningioma. A craniotomy with removal of the macroscopic tumor was performed. Pathology disclosed an anaplastic hemangiopericytoma of the tentorium cerebelli, grade III of the World Health Organization system. The patient was proposed for RT on the lesion with a margin, with a total dose of 60 Gy/30 fractions/6 weeks. Co-registration of MRI to planning-CT was performed for treatment planning. The patient was under steroid therapy during the length of the treatment, with no significant side effects. The MRI performed 2 month post-therapy revealed a shrinking of the mass and the patient was maintained in surveillance. Subsequent MRIs showed similar findings. Current FU reaches 2 years.
Conclusion: Anaplastic hemangiopericytoma is a rare neoplasm in which RT is an important part in patient treatment. This case illustrates how RT can play a significant role after surgery in the loco-regional control of the disease.

OP12. THERAPEUTIC APPROACH TO GLIOBLASTOMA TREATMENT VIA INTEGRINS PATHWAYS

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Introduction: Glioblastoma (GBM) represented 50% of the tumors that arise within the central nervous system. GBM is characterized by high proliferation, apoptosis resistance and surrounding brain infiltration. One of the cell signalling pathways that is under research is integrins pathways which may constitute a potential therapeutic target in GBM treatment.
Objectives: The main objectives of this work were to evaluate the effect of Shikonin, an $\alpha v\beta 3$ integrin inhibitor, in glioma motility, proliferation and survival in monotherapy and in combination with Temozolomide (TMZ). To attain these objectives a human glioma cell line, the U-118 cells, was incubated with shikonin in different concentrations during 48 hours, alone and in combination with TMZ. The expression of Integrins was evaluated by western blot using specific antibodies. Cell migration was analysed by the scratch assay. Cell proliferation and apoptosis was determined using BrdU/propidium iodide and annexin V assay, respectively.
Results: Our results showed that U-118 cells express mainly the alpha V, beta 3 and alpha 5 integrin subunits. However, in cells treated with shikonin we observed a decrease in integrin expression which was accompanied by a significant reduction in the glioma cells migration and proliferation and by an increase in apoptosis. Moreover, when we treat glioma cells with shikonin in combination with TMZ, in lower doses comparing with those used in monotherapy, we observed a synergistic effect.
Conclusion: Our results suggest that shikonin may be a potential therapeutic agent in the treatment of GBM in monotherapy and/or in combination with temozolomide.

DIGESTIVE CANCER

OP13. DEVELOPMENT OF A NEW MODEL OF COLORECTAL ADENOCARCINOMA OF THE RECTUM AND SIGMOID COLON WITH HEPATIC AND PULMONARY METASTASES

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Introduction: The animal models are fundamental to study the molecular mechanisms of cancer, and should mimic the characteristics of tumor progression. However heterotopic models do not show metastasis, an important factor in a good cancer model. The most used orthotopic model of colorectal cancer (CRC) is the implantation of human CRC cells/tissue in the animal cecum, however this location represents 30% of CRC, being necessary to establish a new model in the left colon and rectum (most common types).
Objectives: Development of an animal model of left colon and rectum adenocarcinomas with distant metastases.
Methods: Using the three human CRC cell lines (WiDr, C2BBe1 and LS1034) injected (10-14 × 10^6 cells/animal) in RNU rats (n = 25) which underwent a descending colostomy with mucous fistula of the distal colon. To assess tumor progression, nuclear medicine imaging was performed, using ^{99m}Tc-MIBI.
Results: 1) Only the lines WiDr and C2BBe1 developed locally with tumor histological characteristics similar to the human pathology. 2) Only the line C2BBe1 developed pulmonary and hepatic metastases. The imaging studies demonstrated tumor uptake and possible metastasis distance in these cases.
Conclusion: It was possible to obtain for the first time an animal model of CRC with hepatic and pulmonary metastases. This animal model may contribute to a better understanding of the effectiveness of new CRC drugs and molecular targets and new therapeutic options.

OP14. THE ROLE OF NUCLEAR MEDICINE IN THE STUDY OF MULTIDRUG RESISTANCE IN HEPATOCELULAR CARCINOMA

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Introduction: Hepatocellular Carcinoma (HCC) is known to be highly resistant to chemotherapy, which is due in part to overexpression of multidrug resistance proteins (MDR). A method to measure the function of these proteins involves the study of radiolabeled substrate ^{99m}Tc-MIBI uptake. Studies have demonstrated that ^{18}F-FDG uptake is associated with tumor differentiation and the expression

of these proteins in HCC. This study aims evaluate and compare the uptake and retention of ^{18}F-FDG and ^{99m}Tc-MIBI in two human HCC cell lines with different IN expression levels of p53 and to correlate with the expression of three MDR proteins (Pgp, MRP1 and LRP).
Methods: Human HCC cell lines used were HepG2 (wp53) and HuH7 (mp53). Cell suspensions were incubated with 2×10^6 cells/ml with ^{18}F-FDG and ^{99m}Tc-MIBI (25 µCi/ml). Samples of 200 µl were collected at different periods of time which were centrifuged separating the supernatant from the pellet. Activity was measured in a well counter. The retention of ^{18}F-FDG and ^{99m}Tc-MIBI was obtained by incubating the cell suspension with radioisotope during 60 minutes. Thereafter, the cells were centrifuged and medium renewed. The following procedure was similar to the uptake studies. The proteins levels of Pgp, MRP1 and LRP were determined by flow cytometry. To evaluate MDR modulation, retention studies were performed in the presence of verapamil (Pgp inhibitor) prior to incubation with ^{18}F-FDG.
Results: HuH7cell line is one that has higher levels of uptake and retention of ^{99m}Tc-MIBI and also ^{18}F-FDG. The HepG2 cell line has a lower uptake and retention and a higher expression of MRP1. The levels of Pgp and LRP expression are similar for both cell lines. Through studies of modulation was verified, by incubating the cells with verapamil, a occurs considerable increase in ^{18}F-FDG retention.
Conclusion: There is an inverse relationship between MRP1 expressions and uptake and retention of ^{99m}Tc-MIBI and ^{18}F-FDG. Through modulation studies it was found that Pgp has an active role on MDR phenomenon in HCC. The uptake and retention profiles for the two radiopharmaceuticals are similar, showing that the ^{18}F-FDG can be used to study the action of MDR proteins in HCC cells, presented as an alternative to ^{99m}Tc-MIBI.

HEAD AND NECK CANCER

OP15. THE PROMISE OF A NON-INVASIVE METHODOLOGY TO FOLLOW-UP THE PATIENTS TREATED FOR ORAL CANCER - WHICH IS THE IMPACT IN THE CLINICAL PRACTICE?

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Introduction: Patients with oral cavity carcinoma have a high morbidity and mortality rates mostly because the diagnostic of these tumors occurs at an advanced stage and also due to the predisposition to local recurrence and second primary tumors. Despite being simple to examine the oral cavity, the clinical detection of primary tumors and also the recurrences remains very challenging. The main goal of the present study was the implementation of a non-invasive methodology to perform the follow-up of the patients treated for oral cavity carcinoma. Thus, we intended to combine a non-invasive way of obtaining cancer cells with Multiplex Ligation-dependent Probe Amplification (MLPA) technique in order to detect the genetic imbalances present in these samples.
Methods: Tumor cells were collected by scrapping the surface of the tumor in 16 patients with oral cavity carcinoma diagnosis. Biopsies were also obtained of the tumor from the same patients. To validate this non-invasive methodology we compare the MLPA results obtained in the material scraped and in the tumour biopsy. Once achieved this validation, we performed the patients' follow-up using DNA extracted from cells scraped in the region where the tumor was initially localized, during the routine medical appointments, even if the physician did not detect any suspicious lesion by visual examination. The DNA from healthy donors was used as control.
Results: In general, the results obtained in the samples collected by a non-invasive way, scrapping before the tumor removal, were in agreement with those of the tumor biopsies from the same patients. In this way, we assume that the cells scraped in the region where the tumor was initially localized are good material to follow these patients. During the first year of patients' follow-up, this new methodology seems to be able to detect genomic imbalances even at a low-level, which makes it important to monitor these patients. In this follow-up period of time, we detected in few patients some imbalances already identified in the primary tumors, which could be an indication of high risk of relapse.
Conclusion: Using the MLPA methodology in the tumor tissue and in scraped cells collected in the presence of the tumor (before surgery) it was possible to validate this non-invasive way of collecting samples and opened new possibilities in patients' follow-up after surgery. Our preliminary results in terms of follow-up are very promising and seem to be a window of hope for patients and clinicians.

OP16. HEAD AND NECK CANCER: FROM THE GENOTYPE TO PHENOTYPE AND THEIR IMPLICATIONS

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Introduction: Worldwide, Head and neck (HN) cancer is the 8th most prevalent type of cancer in men. Tobacco smoking and high alcohol consumption are the main etiologic factors for the development of this neoplasm. Although the easy assessment of the HN region, these tumors are usually diagnosed at an advanced stage with complicated outcome. These tumors have a huge biologic variability with small tumors presenting a bad outcome and the larger ones sometimes presenting a better prognosis. Knowledge of the molecular biology of these tumors acquired in recent years has not been associated with significant improvements in healing and survival rates, mainly due to tumor heterogeneity, co-morbidities and a high rate of synchronous and second primary tumors. A more comprehensive understanding of the molecular genetics of the HN tumors, including the oral cavity, could introduce new biomarkers with potential application on diagnosis, staging, monitoring and prognostication. In HN carcinogenesis has been described several chromosomal imbalances, namely gains at 3q, 5p, 7p, 8q, 9q, 11q13, 20q and losses at 3p, 9p, 5q, 8p, 13q, 18q, 21q (Gollin. Head Neck. 2001.23(3):238-53). The aim of this study was to identify genomic alterations on our cohort that will involve therapeutic and clinical decisions.
Methods: Biopsies of tumor were acquired from 28 patients. The Multiplex Ligation-dependent Probe Amplification was conducted using 4 probe panels specific for tumor samples. Healthy donors were used as controls.
Results: In general, we identified imbalances in almost all chromosomes. The results are consistent with the published literature. From the 28 patients analysed, 12 patients developed

cervical metastases, 5 developed lung metastases and 7 died. In patients who developed cervical metastases, we found in almost 67% of the cases, gains in 6p21 (VEGF), 8q24 (RNF139, EXT1, MYC, PTP4A3) and 11q13 (CCND1, FGF3, CTTN) as well as losses in 8p22 (TUSC3). Those who developed lung metastases showed in 80% of the cases gains 8q11.2 and 8q24.1 (PRKDC, EXT1 and RNF139, respectively).

Conclusion: In dead patients, the genetic pattern combines both cervical and pulmonary metastatic that is logic if the cause of dead is the tumor. It will be necessary to study a larger cohort to reinforce these conclusions. We hope, despite the variability referred, to be able to establish a genomic profile as guidance at different stages of treatment protocols for these patients.