into caspase 3 involvement on this cell-death process and, furthermore, to characterize the role of other apoptogenic molecules such us cytochrome c, p53 or AIF (apoptosis inducing factor) into this phenomenom. On this regard, active caspase 3 levels were studied by western-blot and also by measuring enzymatic activity of this caspase at different time points on leukemic cells incubated below 4 ºC. Additionally, we have studied the timing of cytochrome c release and the expression patterns of p53 and AIF on cytosolic, mitochondrial and nuclear fractions. In summary, there are different expression and/or release patterns of apoptogenic molecules on resistant versus sensitive leukemic cells, which correlates with the cell death time course observed for each one of these leukemic cells. The study of the signalling molecules implicated on cold stress-induced cell-death is fundamental on the design of new approaches that allow a better understanding to eliminate drug-resistant tumours.

572 MDR modulation in adenocarcinoma cell lines: nuclear medicine as an important approach

J. Casalta-Lopes[1], A.M. Abrantes[2], J. Rio[3], M. Laranjo[1], B. Oliveiros[1], C. Gonçalves[4], A.B. Sarmento-Ribeiro[4], M.F. Botelho[5]. *[1]Instituto Biofisica/Biomatematica IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [2]Instituto Biofisica/Biomatematica CIMAGO IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [3]ICNAS Universidade de Coimbra, Instituto Biofisica/Biomatematica FMUC, Coimbra, Portugal, [4]Faculdade de Medicina, Instituto Bioquímica, Coimbra, Portugal, [5]Instituto Biofisica/Biomatematica CIMAGO IBILI-FMUC, ICNAS Universidade de Coimbra, Coimbra, Portugal*

Background: One of the major setbacks to chemotherapy is multidrug resistance (MDR), characterized by cross-resistance to several drugs. It can occur due to overexpression of efflux pumps such as P-glycoprotein (Pgp), multiple resistance-related protein 1 (MRP1) and lung resistance-related protein (LRP). They have different extrusion mechanisms but confer resistance to similar substrates. L-buthionine-sulfoximine (BSO) inhibits glutathione synthesis and can be used as blocker for MRP1. Verapamil is a known substrate for Pgp, modulating its activity. In this study we aim to compare transport kinetics for sensitive and resistant human colorectal adenocarcinoma cell lines, in the presence and absence of verapamil and BSO, through 99mTc-MIBI.
Methods: Pgp, MRP1 and LRP expression was evaluated in resistant (LS1034) and sensitive (WiDr) human colorectal adenocarcinoma cell lines using flow-cytometry. Pgp expression was also analyzed using western blotting techniques. Cellular transport kinetics was analyzed in the presence and absence of verapamil and BSO. Retention studies were performed after cell incubation with those drugs, for different time intervals (10 and 60 minutes) and concentrations (10, 25, 50 and 100 μM) with 99mTc-MIBI. Cells were incubated for 60 minutes, washed after and resuspended in fresh medium. Samples were collected and cell metabolism stopped at different time-points in order to obtain retention percentage, measured by gamma-counting adjusted for 140 keV. Retention studies were also performed using LigandTracer® Yellow (Ridgeview Instruments AB, Uppsala-Sweden), an equipment that enables real-time measurements and obtains continuous retention curves. Data was analyzed using appropriate software.
Results: Pgp and MRP1 expression was significantly higher ($p < 0.05$) in resistant cells when compared to the sensitive ones, although LRP was also expressed. Western blotting analysis confirmed flow-cytometry results. 99mTc-MIBI retention percentage was significantly higher ($p < 0.05$) in the resistant cell line when compared with the sensitive one for all time-points. In resistant cells incubated with MDR modulators there were no statistically significant differences ($p > 0.05$) when all points of the retention curves are considered; however there are differences among the MDR modulators used, for the first minutes.
Conclusions: The data obtained until now suggest that these modulators must be used immediately before the cytotoxic drug is administrated.

573 Does GLUTs expression influence 18F-FDG uptake? Study in breast cancer cell lines

A.M. Abrantes[1], M. Martins[2], M.C. Rodrigues[3], S. Tavares[4], C. Mamede[5], C. Gonçalves[6], B. Oliveiros[7], A.B. Sarmento-Ribeiro[6], A. Rodrigues[3], M.F. Botelho[8]. *[1]Instituto Biofisica/Biomatematica CIMAGO IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [2]Serviço Medicina Nuclear HUC, Instituto Biofisica/Biomatematica IBILI-FMUC, Coimbra, Portugal, [3]ICNAS, Universidade de Coimbra, Coimbra, Portugal, [4]Instituto Biofisica/Biomatematica IBILI-FMUC FCTUC, Universidade de Coimbra, Coimbra, Portugal, [5]Instituto Biofisica/Biomatematica IBILI-FM Universidade de Coimbra, Universidade da Beira Interior, Coimbra, Portugal, [6]Instituto Bioqímica, Faculdade Medicina Coimbra, Coimbra, Portugal, [7]Instituto Biofisica/Biomatematica IBILI, Faculdade Medicina Coimbra, Coimbra, Portugal, [8]Instituto Biofisica/Biomatematica CIMAGO IBILI-FMUC, ICNAS Universidade de Coimbra, Coimbra, Portugal*

Background: Positron emission tomography (PET) uses the radiolabeled glucose analogue ^{18}F-FDG to detect glycolysis in cancer cells. ^{18}F-FDG uptake by cancer cells showed high value which allows diagnosis, staging, and detection of recurrence and evaluation of response to therapy in several malignancies.
Breast cancer is the most common malignancy among women with an increasing prevalence and is potentially curable when diagnosed early and the treatment is optimized. For the appropriate hormonal therapy the expression of estrogen and progesterone receptors is essential. The presence of the receptor HER2/neu was recently introduced as a new predictive marker of prognosis.
Breast cancer is of considerable variability in the uptake of ^{18}F-FDG, which results in different sensitivity and specificity, which in turn interferes on evaluation. The ^{18}F-FDG enters in cells through the same mechanisms of membrane transport of glucose, the glucose transporters (GLUT). Among the GLUT isoforms, the GLUT-1 and GLUT-3 overexpression is one of the mechanisms responsible for the increased utilization of glucose by tumour cells.
Aims: In this context, the main objective of this study is to determine the ^{18}F-FDG uptake in two cell lines of breast cancer with different expression of hormonal receptors and overrexpression of HER2 gene and setting eventual correlation with the expression of GLUT-1 and GLUT-3.
Material and Methods: Two different cell lines of human breast cancer, MCF-7 (estrogen and progesterone receptors positive) and HCC1806 (triple negative) were used. ^{18}F-FDG uptake for both cell lines was obtained for different times. The expression of GLUT-1 and GLUT-3 were analyzed by flow cytometry for two cell lines.
Results: When analyzed GLUT-1 and GLUT-3 expression by flow cytometry, it was found that HCC1806 cell line had higher expression than MCF-7. The ^{18}F-FDG uptake was significantly higher in MCF-7 cell line than HCC1806.
Conclusions: Despite the expression of GLUT-1 and GLUT-3 isoforms be responsible for ^{18}F-FDG uptake we verified a negative correlation between expression of glucose transporters and ^{18}F-FDG uptake.

574 Cellular prion-heat-shock organizing protein interaction as a new therapeutic target for glioblastomas

N.G.T. Queiroz-Hazarbassanov[1], M.H. Lopes[1], I.W. Cunha[2], S.M. Oba-Shinjo[3], S.K.N. Marie[3], M.D. Inda[4], V.R. Martins[1]. *[1]Hospital A. C. Camargo and Ludwig Institute for Cancer Research, Cellular and Molecular Biology Laboratory, São Paulo, SP, Brazil, [2]Hospital A. C. Camargo, Pathology Department, São Paulo I SP, Brazil, [3]School of Medicine of the University of São Paulo, Department of Neurology, São Paulo, SP, Brazil, [4]Ludwig Institute for Cancer Research, Laboratory of Tumour Biology, San Diego, CA, USA*

Malignant gliomas are the most common primary brain tumours and are nearly uniformly fatal, without yet an effective therapy. Previous data from our group have shown that cellular prion protein (PrP^C) and secreted STI1/Hop (Stress induced phosphoprotein 1/hsp70-hsp90 organizing protein) interaction induces glioblastoma proliferation. Additionally, real time PCR and immunohistochemistry of clinical specimens from glioblastoma patients demonstrated that STI1/Hop mRNA and protein expression is significantly higher in these tumours than in normal tissues (Mann-Whitney test, $p < 0.05$ and $p < 0.0001$, respectively). The purpose of this study was to block glioblastoma proliferation using an $STI1/Hop_{230-245}$ peptide, which mimics the STI1/Hop binding site at PrP^C to compete PrP^C-STI1/Hop interaction. Human glioblastoma cell line (U87MG) proliferation was evaluated *in vitro* by bromodeoxyuridine (BrdU) incorporation, anti-BrdU based immunofluorescence and total/BrdU positive nuclei counting. Proliferation of U87MG induced by STI1/Hop treatment was abolished by $STI1/Hop_{230-245}$ (ANOVA-Tukey, $p < 0.05$), while a control peptide from STI1/Hop ($STI1/Hop_{61-76}$) had no effect. Furthermore, intratumoural infusion of three different concentrations $STI1/Hop_{230-245}$ in pre-established U87MG orthotopic xenograft tumours in nude mice delayed tumour growth, compared to saline and control peptide (ANOVA-Kruskal-Wallis, Dunns post test, $p < 0.01$). Immunofluorescence analysis of xenografts using anti-Ki67, anti-caspase 3 and anti-CD31 antibodies revealed that $STI1/Hop_{230-245}$ treatment decreased tumour proliferation and increased apoptosis *in vivo* ($STI1/Hop_{230-245}$ vs. control peptide, t test, $p < 0.0001$ and $p = 0.0002$, respectively), although no change was observed in angiogenesis. Thus, we suggest PrP^C-STI1/Hop as a novel molecular target for glioblastomas and $STI1/Hop_{230-245}$ as a promising candidate for cancer therapy.

575 Alpha-secretase and neprilysin enzyme activities are decreased in renal cell carcinoma

N. Erin[1], T. Ipekçi[2], B.K. Akkaya[3], M. Baykara[2]. *[1]Akdeniz University School of Medicine, Department of Clinical Pharmacology and Health Sciences Laboratories, Antalya, Turkey, [2]Akdeniz University School of Medicine, Department of Urology, Antalya, Turkey, [3]Akdeniz University School of Medicine, Department of Pathology, Antalya, Turkey*

Background: Renal cell carcinoma (RCC) is a malignancy which does not response well to conventional chemotherapy and radiotherapy. Hence, the identification of molecules involved in the development and progression of