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

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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

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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

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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

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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

this disease is of high priority. ADAMs are a family of metaloproteases and increased expression of ADAM9, ADAM10 and ADAM17 was associated with tumour progression in some but not in all studies. Similarly how change in neprilysin level, a peptidase involved in hydrolysis of neuropeptides, affects tumour formation and progression is not clear. These discrepancies may reflect the fact that chances in protein level do not always correlate with the functional changes. Hence determination of functional changes is crucial to really understand how the candidate molecules affect tumour formation and metastasis.

Material and Methods: We here examined the chances in alpha-secretase activity as an assay for ADAM9, 10 and 17 function as well as neprilysin activity in freshly frozen RCCs. Adjacent renal tissue ~2 cm distant to primary tumour was also used. RCCs were obtained from 45 patients. The percent of clear cell, papillary and chromophobe RCCs were 58; 16 and 11 respectively. The rest were other types.

Results: We also obtained renal tissues from patients who underwent surgery for nephrolithiasis. We found that both alpha-secretase and neprilysin activity was markedly decreased (78% and 57% respectively) in tumour tissue compared to tumour-free neighboring renal tissue ($p < 0.0001$ paired t-test). Enzyme activity did not differ markedly among different histological subtypes. Renal alpha-secretase activity of RCC patients were significantly higher (2.4 times) compared to renal tissue of nephrolithiasis patients. In contrast, renal neprilysin activity of chromophobe RCC patients were decreased significantly (44%) compared to nephrolithiasis patients.

Conclusion: These results demonstrated for the first time that neprilysin and alpha-secretase activities are decreased in RCC and further studies are required to determine therapeutic significance of these findings.

576 Expression of TASK-1 and TASK-3 channels in non-small cell lung cancer

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Background: Lung cancer is the leading cause of cancer deaths overall in the world. Survival times are poor, despite advances in therapy. Two-pore domain K^+ (K2P) channels are a family of ion channels expressed in pulmonary arteries and lung airway epithelial cells. K2P channels conduct background K^+ current. The K2P channel TASK-3 (KCNK9) has been implicated in cancer growth, but nothing is known about the role of the acid and hypoxia sensitive TASK-1 (KCNK3) K^+ channel in cancer.

Materials and Methods: Expression of TASK-1 and TASK-3 mRNA was analyzed in non-small cell lung cancer (NSCLC) surgery specimens and normal lungs from twenty-four patients as well as in A549, NCI-H358 and A427 NSCLC cell lines. Immunostaining for TASK-1 in NSCLC tissue and patch-clamp measurements of leak (background) K^+ currents were performed.

Results: TASK-1 mRNA expression was present in 24/24 NSCLC samples, TASK-3 mRNA was found at detectable levels in 21/24 samples. The median level of TASK-3 mRNA was about 160 times lower compared to the median level of TASK-1 mRNA. However, TASK-1 mRNA levels were significantly reduced in tumours, compared to lungs ($P = 0.00002$). Using confocal microscopy TASK-1 immunoreactivity was found to be localized perinuclearly and in the membrane of tumour cells in NSCLC tissue. In the investigated cell lines the highest TASK-1 expression was found in A549 cells. When K^+ currents were analyzed in A549 cells, a typical non-inactivating, hypoxia and acid sensitive current was detected. The current was reduced and the membrane was depolarized by the TASK-1 inhibitor anandamide and by siRNA mediated knockdown of TASK-1.

Conclusions: The study shows for the first time that TASK-1 is functionally expressed in NSCLC and mediates hyperpolarization of tumour cells. The role of TASK-1 in NSCLC progression is unkown. However, since TASK-1 was detected at clearly lower levels in NSCLC compared to the parental tissue, NSCLC carcinogenesis might be accompanied by a reduction of tumour cell TASK-1 current with consequent membrane depolarisation and inhibition of K^+ efflux.

577 Endothelial cells increase invasiveness of glioblastoma cells and protect them from apoptosis

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Background: Glioblastoma is the most malignant tumour of the central nervous system and the average survival of glioblastoma patients is only 14 months. The key reason for the lack of successful therapy is the infiltration of single tumour cells into the surrounding brain parenchyma, preventing complete resection. The infiltrating cells are also more resistant to chemo- or radiotherapy.

Methods: Co-culture experiments were carried out in modified Boyden chambers. Protease expressions were measured by RT-PCR and their role was confirmed using synthetic inhibitors. The level of apoptosis was measured on flow cytometer.

Results: Invasion of glioblastoma cells often occurs along blood vessels suggesting an important interaction between both. In the present study we demonstrate that co-culture of U87 glioblastoma cells with HMEC-1 endothelial cells markedly increases the invasiveness of the tumour cells [1]. This enhanced invasiveness correlates with increased expression of MMP-9 in both U87 and HMEC-1 cells and increased expression of cathepsin B in U87 cells only. Being up-regulated and secreted by both cell lines, MMP-9 had a higher impact on tumour cell invasion than cathepsin B. Cathepsin S was also up-regulated in the co-culture, but its role in invasion was not confirmed in our experiments. HMEC-1 exerted their invasion promoting effect on U87 cells mostly through secretion of SDF-1. SDF-1 inhibition by neutralizing antibody blocked the increase in U87 cell invasion, most likely via down-regulation of MMP-9 in U87 cells. HMEC-1 endothelial cells also protect glioblastoma cells from apoptosis. The induction of apoptosis in glioblastoma cells after staurosporine and TNF-a treatment was significantly lower in the co-culture, compared to U87 culture alone. The expression of cathepsin L, known to oppose apoptotic efficacy of these agents in U87 cell (2), correlated inversely with the level of apoptosis.

Conclusion: Taken together, our study suggests that tumour cells may be attracted and protected by endothelial cells in normoxic conditions and underlines the importance of SDF-1, cathepsins B and L as well as MMP-9 in the cross-talk between these cells. It contributes towards a better understanding of glioblastoma and endothelial cell interactions, needed to improve various treatment modalities.

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578 TGFB2 and NTF3 stromal chemokines that correlate with colorectal cancer progression

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Background: Interaction between cancer and stromal cells play critical roles in tumour development and progression. This study wanted to assess relevant stromal genes in cancer progression. Identification of a stromal cancer progression signature could provide new therapeutic targets.

Methods: Normal colonic fibroblasts adjacent to the tumour (NCF) and paired CAFs from primary tumour (CAFpt) were obtained from ten colorectal cancer patients specimens. Six liver CAFs were also isolated from metastatic patients from CRC. RNA was hybridised in Affymetrix GeneChip Human Gene 1.0 ST Array. *SAM* method (*Significance Analysis Microarrays)* was used to analyse differential expression between NCF, CAFpt and liver CAFs. Gene-annotation enrichment analysis was carried out using DAVID Bioinformatic Resources. Conditioned media (CM) from these fibroblasts were used to perform functional assays in different colon cancer cells lines (DLD-1, SW620, SW480, and SW1116). A tumourogenic assay in vivo was also carried out.

Results: CM from NCF and CAFpt increased proliferation of colorectal cancer cell lines to a greater extent than cultured with CM from liver CAFs. However, CAFs from liver metastases increased motility, migration and invasiveness of colorectal cancer cells. Comparison of transcriptomic data from NCF and paired CAFpt results in 48/28869 genes overexpressed in CAFpt (P and FDRq-value <0.05; 15/28869 genes considering FDRq-value = 0; highlighting TNFSF4, NTF3, ST6GALNAC5, TGFB2, GALNTL4), and 103 genes infraexpressed (28/28869 genes considering FDR q-value = 0; FGF13, IL1R1, IL33, PTGS2, ATP8B4). Comparing CAFpt and metastatic CAFs, 99 genes were overexpressed in colon CAFpt (FDRq-value = 0; FBN2, BMP6, PDPN, CXCL14, ITGB3) and 52 genes in hepatic CAFs (FDRq-value = 0; highlighting PDGFA, SPINK1, MMP1, VEGFA, ICAM2).

Interestingly, when we compared at same time the three populations (NCF, CAFpt and hepatic CAF) taking into account genes whose expression matched a linear regression, 19 genes/28869 (FDRq-value = 0) were overexpressed in metastatic CAFs (AFAP1, TRAF4, TGFB2, NTF3, TNFSF18) and 76 were infraexpressed (FDRq-value = 0; FGF13, TGFBR3, IL1R1, AKR1C2, TNFSF10). We validated by RT-PCR these gene sets in a independent set of fibroblasts (n = 41). We are validating TGFB2, NTF3 and IL1R1 by IHC in a series of 40 matched normal colonic mucosa, primary tumour, normal liver and liver metastasis from the same patient (work in progress).