ErbB2 expression with specific siRNA blocked the PGE_2-induced amplification of cyclin D1 expression and DNA synthesis in response to EGF.
Conclusion: The results suggest that the upregulation by PGE_2 of the mitogenic response of hepatocytes to EGF may at least in part be mediated by increased expression of ErbB2.

488 Quantitative proteomics reveals secreted factors governing enhanced motility in rat C6 glioma cells expressing connexin43

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Background: Glioblastoma multiforme is a devastating tumour of the brain demonstrating higher rates of motility and invasion potential. Recent evidence has implicated the gap junction protein connexin43 (Cx43) in the motility of brain tumour cells. Supporting these findings, we also observed a correlated increase in motility of C6 glioma cells over-expressing Cx43 (C6−13 cells) compared to their wild-type counterparts (C6 cells). Since migration of tumour cells involves the secretion of proteolytic enzymes and cytokines, we tested the effect of the conditioned medium from C6−13 cells and observed that it increased the migration capacity of C6 cells up to the C6−13 cell level. In order to understand the molecular pathways associated with such a process, we have undertaken a proteomic approach to identify and quantify proteins secreted within the conditioned media of wild-type C6 cells and C6−13 cells.
Materials and Methods: Proteins isolated from media of 80% confluent C6 or C6−13 cell cultures were isotopically labeled at the peptide-level by reductive dimethylation and analyzed on a high performance liquid chromatograph hyphenated to a high-resolution linear trapping quadrupole-Orbitrap mass spectrometer by using Xcalibur software. Fragments spectra were identified using Mascot (v.2.2, Matrix Science) and quantitative ratios were extracted using MSQuant (http://msquant.sourceforge.net/).
Results: Differential analysis revealed, within the conditioned media of C6−13 cells, a significant up-regulation of secreted proteins involved in cell migration and known as markers of glioma aggressiveness. Such proteins were either cytokines (small inducible cytokine A2, osteopontin, latent TGF-B binding protein-1, lectin galactoside-binding soluble 3 binding protein), proteolytic enzymes (MMP3, cathepsins B and L1) and extracellular matrix compounds (collagen alpha-1 (VI), SPARC, tenascin-C and fibronectin). However, some extracellular matrix compounds were found to be decreased in the C6−13 culture medium (elastin microfibril interface 1, various procollagens) as a possible direct consequence of the action of the oversecreted proteolytic enzymes.
Conclusion: Findings presented in this study provide insights into enhanced cell motility linked to Cx43 expression and the molecular cues associated with the migration of tumour cells. Determining how Cx43 triggers the secretion of such diffusible factors involved in glioma cell invasion may lead to new therapeutics considerations.
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489 New animal model in colorectal cancer

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Background: To gain confidence in the validity of animal models research is essential to unequivocal quality and convincing data. Colon cancer is one of the most prevalent tumours in the world. Despite this, only in 2007 was presented a colon adenocarcinoma model in null mice. In this model, cancer cells were inoculated in animal cecum. A few considerations about this model should be made. Firstly, colorectal cancer is less usual in cecum, actually for this tumour type the most prevalent localization is distal colon. Secondly, inoculation in serosa layer in detriment of colonic mucosa where these tumours originate and, finally, maintenance of impossibility of monitoring tumour growth over time as an additional disadvantage. The aim of this study is to present new adenocarcinoma animal model in left colon that allows us monitoring tumour growth.
Material and Methods: Colon exclusion was made and distal fisula was kept open. Adenocarcinoma cells (WiDR) was inoculated in mucosa fistula after normal bowel function return. Neoplastic growing was monitored daily. Scintigraphic method was performed to tumour detection.
Results: After 4 days tumour growing was observed. Fifteen days after cells inoculation, tumour detection was possible to use molecular imaging, ten minutes after 99mTc-MIBI administration. Macroscopy demonstrated tumour invasion in proximal colon and it partial lumen occlusion. Microscopy demonstrated undifferentiated tumour with infiltration in all colon layers.
In conclusion this new colorectal cancer animal model is feasible and allows measuring it external growth and monitoring by ^{99m}Tc-MIBI scintigraphy.

490 Changes in expression profiles of apoptosis, invasion, metastasis, angiogenesis, transcription factors, cell cycle control and tumour supressor genes in nilotinib treated chronic myeloid leukemia cells

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Background: Chronic myeloid leukemia (CML) is a hematological malignancy arising from a reciprocal translocation between long arms of chromosomes 9 and 22. The resulting BCR/ABL fusion protein is a strong oncogenic protein that regulates cell growth and proliferation, apoptosis and senescence, migration and adhesion. Imatinib was the first tyrosine kinase inhibitor for the treatment of CML. Although there were significant hematologic and cytogenetic responses to imatinib, resistance cases were observed in patients during treatments and this was the major drawback of imatinib treatment. After identification of the mechanisms of imatinib resistance, a more effective anticancer agent, nilotinib, was developed and started to be used for the treatment of Philadelphia chromosome positive hematological malignancies.
Aims: In this study, we aimed to examine the molecular mechanisms of nilotinib-induced cell death in addition to inhibition of BCR/ABL in K562 chronic myeloid leukemia cells.
Materials and Methods: Antiproliferative effects of nilotinib were determined by XTT cell proliferation assay. Increasing concentration of Nilotinib (20 and 50 nM) were applied to K562 cells. After 72 hours incubation, total RNAs were isolated and converted to cDNA. Changes in expression levels of 84 genes involved in apoptosis, cell cycle, senescence, adhesion, invasion, metastasis, angiogenesis, transcription factors, and signal transduction molecules were examined by PCR array.
Results: There were 40 and 55% decreases in proliferation of K562 cells in response to 20 and 50 nM nilotinib, respectively, as compared to untreated controls. Gene expression results revealed that 50 nM nilotinib application resulted in more than 4-fold increases/decreases in expression levels of 41/6 genes as compared to untreated controls and normalized to housekeeping genes. On the other hand, lower concentration of nilotinib, 20 nM, increased/inhibited expression levels of 2/22 genes more than 2-fold comparing to untreated controls and normalized to housekeeping genes.. Nilotinib induced expression levels of apoptotic (Bax, Serpin5B, GZMA, TNF, TNFRSF25, APAF1) cell cycle controlling (CDK2, CDKN2A, MDM2), and inhibitor of metastasis (TIMP1, TIMP3) genes and decreased expression levels of growth inducing (AKT1, IGF1, MYC, NFKB, MAP2K1, PLAU), antiapoptotic (SNCG, SYK), metastatic (MMP1, MMP2, ITGB5, ITGA3) and angiogenic (IL-8, ANGPT2) genes. The highest increases were observed in apoptotic TNF and GZMA genes while the highest decreases were observed in growth inducing MAP2K1 and PLAU genes.
Conclusion: In this study, we demonstrated the molecular mechanisms of nilotinib induced cell death in addition to inhibition of oncogenic BCR/ABL protein. More importantly, we have also showed for the first time that nilotinib also has the potential to inhibit metastasis and angiogenesis through manipulating metastatic and angiogenic genes.

491 J7, a methyl jasmonate analogue, enhances TRAIL-mediated apoptosis through reactive oxygen species generation

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Background: The jasmonates are fatty-acid-derived cyclopentanones that occur ubiquitously in the plant kingdom and they serve as natural bioregulators and are involved in plant intracellular signaling and defense in response to injury. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is known to induce apoptosis in cancer cells but spare most normal cells. However, its effect (s) is limited in some types of cancer cells, including HepG2 human hepatocarcinoma cells. In the present study, we showed that treatment

with TRAIL in combination with subtoxic concentrations of a synthetic methyl jasmonate derivative (J7) sensitized TRAIL-mediated apoptosis in HepG2 cells.
Material and Methods: HepG2 cells were incubated with TRAIL, J7, caspases inhibitor and ROS scavenger NAC. Growth activity was assessed using a MTT assay, the effects of apoptosis induction by TRAIL and J7, and the regulatory mechanisms were studied by DAPI staining, flow cytometry, caspase assay kits and Western blot analyses.
Results: Combined treatment with J7 and TRAIL induced rapid apoptosis in TRAIL-resistant HepG2 cells and effectively induced Bid cleavage and down-regulation of IAP family proteins, leading to the activation of caspases, and cleavage of poly(ADP-ribose) polymerase. The reactive oxygen species (ROS) were significantly up-regulated in cells following exposure to TRAIL and J7, indeed, the pre-treatment of ROS scavenger by NAC attenuated J7 plus TRAIL-induced apoptosis. Furthermore, pretreatment an ERK-specific inhibitor, PD98059, and a p38-specific inhibitor, SB203580, showed increased sub-G1 phase DNA content and activation of caspases in TRAIL and J7 induced apoptosis.
Conclusions: The use of TRAIL in combination with subtoxic doses of J7 may provide an effective therapeutic strategy for safely treating some TRAIL-resistant hepatoma cancer cells.

492 Role of Bcl-2 family members on cold stress-induced cell-death in multidrug resistant leukemic cells

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The family of Bcl-2 proteins plays a key role as regulators of apoptosis. In healthy cells these proteins are maintained in a basal latent state, but in response to apoptotic stimuli they become activated through a variety of mechanisms involving post-translational modification or transcriptional activation. Here, we have undertaken the study of Bcl-2 family members involved on cold stress-induced cell death on resistant leukemic cells. Thus, we have focussed on Bcl-xL and Bcl-2, as anti-apoptotic members and Bax (belonging to the multi-domain or Bax subfamily) and Bad (belonging to the BH3-only subfamily) as pro-apoptotic members. By using western-blot techniques, we have found that acquisition of MDR phenotype by leukemic cells is accompanied by changes on the expression of Bcl-2 family members. Specifically, Bcl-xL levels diminish on MDR versus sensitive cells, while Bcl-2 protein expression increases on resistant cells in comparison to their sensitive counterparts. Furthermore, silencing experiments demonstrate the protective role of Bcl-xL on leukemic cells. Additionally, alterations in subcellular location have been observed for Bcl-2 proteins. These events lead to an increase on the outer mitochondrial membrane permeability followed by the translocation of cytochrome c and other apoptogenic factors. Together, these findings demonstrate that during the process of drug resistance, leukemic cells undergo alterations on Bcl-2 family members expression, among others. This fact could explain viability differences on sensitive *versus* resistant leukemic cells under stress conditions.

493 Gene expression profiles in resveratrol-induced cell death in acute promyelocytic leukemia cells

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Background: Resveratrol, (3,5,4′-trans-trihydroxystilbene) is a natural product found in plant constitutes such as grape skin. It has shown significant cytotoxic and apoptotic effects on various types of cancer cells with no harm to normal healthy cells. Resveratol inhibits tumour initiation, promotion, and progression. However the mechanisms of resveratrol induced cell death is not well-known. We have previously showed cytotoxic and apoptotic effects of resveratrol on acute premyelocytic leukemia (APL) cells. APL is a hematological malignancy characterized by increased number of clonal population of hematopoietic progenitor cells.
Aims: In this study, we aimed to show molecular mechanisms of resveratrol induced apoptosis by examining the changes in expression profiles of human cancer signalling pathway genes in HL60 APL cells exposed to resveratrol.
Methods: Effective concentrations of resveratrol on HL60 cells were determined by XTT cell proliferation assay. Total RNAs were isolated from HL60 cells exposed to 10 and 50 μM resveratrol, converted to cDNA, and changes in expression levels of 84 genes involved in apoptosis, metastasis, angiogenesis, invasion, adhesion, tumour supressors, and transcription factors by PCR array.
Results: Resveratrol has shown antiproliferative effect on HL60 cells in a dose dependent manner. There were 15 and 45% decreases in cell proliferation in response to 10 and 50 μM resveratrol in HL60 cells as compared to untreated controls, PCR array results demonstrated that there were more than 3-fold increase in expression levels of 24 and 36 genes in HL60 cells treated with the same concentrations of resveratrol as compared to control, respectively. On the other hand, there were 6 genes whose expression levels were decreased more than 4-fold in response to 10 and 50 μM resveratrol, respectively. We observed significant increases in expression levels of p53 in a dose-dependent manner which is not detected in control group. The most significant increases were observed in apoptotic genes (e.g. Bax, Tert), and decreases were observed in antiapoptotic genes (e.g. Bcl-2). Although, there were increases in expression levels of certain growth factor, antiapoptotic and metastatic genes, our whole data demonstrated that these concentrations of resveratrol inhibited cell growth and induced apoptosis in HL60 cells.
Summary and Conclusions: In this study the mechanisms of resveratrol-induced apoptosis were demonstrated in detail. This *in vitro* data by being supported with clinical data may open the way of the potential use of resveratrol for acute premyelocytic leukemia patients.
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494 Clinical significance of lymphangiogenesis in molecular types of invasive breast cancer

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Background: In last years, there were accumulated a lot of data that support the crucial role of lymphangiogenesis in the development of lymph node metastases (LNM) in breast cancer. The expression of lymphangiogenic molecules and lymphatic microvessel density (LMVD) in molecular types of breast cancer were less investigated. In the present work we investigated the relationships between LMVD, VEGF-C and VEGFR-3 expression, molecular types, and clinico-pathological factors of prognosis in breast cancer.
Material and Methods: There were studied 106 patients with invasive breast cancer and 49 had LNM. Cases were stratified according the molecular classification, based on the immunohistochemical expression of estrogen receptor, progesterone receptor, HER2 protein, cytokeratin 5, p53, Bcl-2 and epidermal growth factor receptor. Additional slides were stained for VEGF-C, VEGFR-3, and D2−40. VEGF-C and VEGFR-3 were scored using an intensity/percent-based system, and LMVD was estimated using Weidner's method. Lymphatic vessels (LVs) were counted in the peri- and intratumoural areas.
Results: VEGF-C was strong or moderate positive in 64 cases, and weak and negative in 42 cases. Significant correlation was found with LNM and grade of the tumour (G), but not with the molecular type of carcinoma, excepting for the HER2 type. LMVD was evaluated in the peri- (LVs found in all the cases, range 0.6 to 15.3/×200) and intratumoural (LVs found in 57 cases, range 0 to 7.6/×200) area. Intratumoural but not peritumoural LMVD correlated with LNM, VEGF-C. No correlation was found between LMVD and tumour stage and grade. Higher LMVD were found in HER2 and Luminal B types, and lowest in basal-like carcinoma. Overexpression of VEGF-C was associated with VEGFR-3 expression in the endothelium. Additionally, VEGFR-3 was expressed by tumour cells in 52% of the cases and in 26% by stromal cells. A strong correlation was found between VEGFR-3/VEGF-C expression and LNM.
Conclusion: Our data showed that a differential expression of VEGF-C and VEGFR-3, and different values of LMVD are found in the molecular types of breast cancer. This suggests the prognostic role of these markers and indicates their potential use as targets for antitumour therapy.

495 Cancer-associated adipocytes exhibit an activated phenotype and contribute to early breast cancer invasion in vitro and in vivo

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Early local tumour invasion in breast cancer results in immediate proximity of cancer cells to mature adipocytes, but the role of these cells in tumour progression has been poorly studied. Using a 2D co-culture system, we demonstrate that tumour cells co-cultivated with mature adipocytes exhibit an increase in invasive capacities *in vitro* and *in vivo*. In turn, adipocytes cultivated with cancer cells exhibit delipidation and decreased adipocyte markers associated to the occurrence of an activated phenotype marked by over-expression of proteases, including MMP11, and pro-inflammatory cytokines (IL-6, IL-1b), IL-6 playing a key role in the adipocyte-dependent pro-invasive effect. The phenotypic changes of adipocytes are observed in human breast tumours using immunohistochemistry and qPCR in a series of adipocytes isolated from adipose tissues obtained either from tumourectomy or mammoplasty. The tumours of larger size and/or with lymph nodes involvement exhibit the higher levels of IL-6 in cancer-associated adipocytes. This new bidirectional crosstalk between adipocytes and breast tumour cells might explain the poor prognosis of breast cancer in obese women that frequently exhibit extended tumours at diagnosis.