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