or pharmacological inhibition of this enzyme induced profound apoptosis, which coupled with significant elevation of intracellular ceramide and loss of sphingosine 1-phosphate. *In vivo*, both docetaxel and camptothecin treatment of fluorescent tumors consistent of PC-3/GFP cells o.t. implanted in nude mice induced primary tumor and lymph nodes' volume regression, markedly docetaxel being twice more potent than camptothecin. These events were coupled to sphingosine kinase inhibition and elevation of ceramide/sphingosine 1-phosphate ratio both in primary tumors and in lymph nodes. Markedly, docetaxel treatment abrogated migration of cancer cells and formation of micrometastases.
Collectively our results show that apoptosis induction by chemotherapy in prostate cancer cells is correlated with sphingosine kinase inhibition. Ability of sphingosine kinase to determine the resistance of cancer cells to chemotherapy might propose its role as a responsive element in proapoptotic signaling. Thus modulation of SK activity and thus of ceramide/S1P balance might find an application in cancer treatment.

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Different expression of tight junction proteins in HCC and metastatic liver tumours

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Background: Tight junction (TJ) proteins have already been found implicated in carcinogenesis. A group of integral membrane proteins – occludin, claudins and junctional adhesion molecules – interact with cytoplasmatic tight junction proteins to integrate diverse processes (e.g. tumour suppression, gene transcription, cell polarity).
Material and methods: Expression of claudins, occludin, junctional adhesion molecule (JAM)-1, -2, -3 and zonula occludens (ZO)-1, -2, -3 was analysed in 15 human hepatocellular carcinoma (HCC) and 15 colorectal metastasis in liver to study TJ in liver malignancies. Gene expression levels were measured by real-time PCR, protein expression was determined by immunohistochemistry and Western blot comparing tumours to surrounding parenchyma and to normal liver samples (7).
Results: ZO-1, -2, -3, JAM-1, -2, -3 and occludin mRNAs were significantly downregulated in HCC compared to normal liver (4.6×; 15.3×; 18.2×; 12.9×; 5.9×; 3.3× and 8.2×) and ZO-2, -3, JAM-2 and occludin mRNAs were also significantly downregulated compared to surrounding tissues (3.4×; 5×; 3.2× and 2.2×). In metastasis claudin-4 was significantly upregulated (12.7×), while ZO-1, -2, JAM-1, -2 and occludin were downregulated (6.4×; 9.6×; 9.4×; 18.6× and 12.1×) with respect to normal liver. Immunohistochemistry basically supported RNA expression data. Claudin-3, -4 and -7 staining were very strong in metastasis, while only scattered weak in HCC. TJ proteins were generally weakly expressed on hepatocytes, while strongly on bile canaliculi and arterioles in normal liver.
Conclusions: HCC and metastasis show different pattern of expression of TJ components. Differences in ZO-3, claudin-3 and -4 could be used for differentiation of the primary and secondary tumour. The origin of metastatic tumour could influence TJ protein expression, especially different organs can be characterized by their claudin expression.
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173 POSTER

Characterization of genes with increased expression in glioblastomas

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Background: In the present study, we have used the gene expression data available in the SAGE database in an attempt to identify *glioblastoma molecular* markers.
Material and methods: Nine SAGE libraries of human glioblastoma (GB), six SAGE libraries of GB cell lines, and five SAGE libraries of normal human brain (NB) were analyzed to compare gene expression in GB with that of NB by accessing SAGE NCBI web site http://www.ncbi.nlm.nih.gov/SAGE and using the search tool cDNA Digital Gene Expression Displayer (DGED) provided by the SAGE Genie database. Northern blot analysis was performed for confirmation of enhanced expression of activated genes in glioblastoma.
Results: Of 129 genes with more than 5-fold difference ($P \leqslant 0.05$) found by comparison of nine *glioblastoma* vs. five normal brain SAGE libraries, 44 increased their expression in *glioblastomas*. High expression of 21 genes in *glioblastomas* as well as in *glioblastoma* cell lines suggested that expression in the bulk tumors was from transformed cells. Increasing of expression of 23 other genes only in *glioblastomas* but not in *glioblastoma* cell lines suggested that expression in the bulk tumors was from macrophages/microglial cells. Many of the latter genes are among of the top transcripts in activated macrophages and are involved in the immune response and angiogenesis.
Conclusion: Since constituent parts of tumor, *primary tumor tissue and microglia*, both participate in the tumor growth and development, all genes with highest levels of expression in glioblastomas can be used as molecular markers in the analysis of malignant progression of astrocytic tumors. Moreover, several of genes overexpressed in glioblastomas, produce extracellular proteins, thereby providing opportunities for clinical application. Further characterization of these genes will allow them to be exploited in molecular classification of glial tumors, diagnosis, prognosis, and anticancer therapy.

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Pramanicin induces apoptosis in Jurkat leukemia cells: a role for JNK, p38 and caspase activation

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The improvement in our understanding of the regulation of the molecular machinery of apoptosis reveals that the suppression of apoptosis in the presence of a proliferative stimulus is critical for tumour development. It has been clarified that new therapeutic approaches based on drug targets in apoptotic pathways will improve the response of patients to "target specific" therapeutic approaches. Pramanicin is a novel anti-fungal drug with a wide range of potential application against human diseases. In the present study, we showed that pramanicin induced apoptosis in Jurkat T leukemia cells in a dose- and time-dependent manner.
Our data reveal that pramanicin induced the release of cytochrome *c* and caspase-9 and caspase-3 activation, as evidenced by detection of active caspase fragments and fluorometric caspase assays. Pramanicin also activated c-jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinases (ERK 1/2) with different time and dose kinetics. Treatment of cells with specific MAP kinase and caspase inhibitors further confirmed the mechanistic involvement of these signalling cascades in pramanicin-induced apoptosis. JNK and p38 pathways acted as pro-apoptotic signalling pathways in pramanicin-induced apoptosis, in which they regulated release of cytochrome *c* and caspase activation. In contrast the ERK 1/2 pathway exerted a protective effect through inhibition of cytochrome *c* release from mitochondria and consequent caspase activation, which were only observed when lower concentrations of pramanicin were used as apoptosis-inducing agent.
These results suggest pramanicin as a potential apoptosis-inducing small molecule, which acts through a well-defined JNK- and p38-dependent apoptosis signalling pathway in Jurkat T leukemia cells. Studies focusing on different cancer cell lines and/or experimental animal models will further extend our understanding of mechanisms involved in apoptotic response to pramanicin and will allow us to better evaluate the anti-cancer potential of this molecule.

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MDGA1, a novel human protein with a functional role related to cellular adhesion

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Background: We have reported the characterization of the novel human protein MDGA1 (MAM Domain containing Glycosylphosphatidylinositol Anchor-1 protein). The deduced polypeptide exhibits structural features found in different types of Cell Adhesion Molecules (CAMs), such as the presence of both immunoglobulin domains and a MAM domain or the capacity to anchor to the cell membrane by a GPI (GlycosylPhosphatidylInositol) motif. *MDGA*1 encodes a 955 aminoacids protein containing an N-terminal signal peptide followed by six immunoglobulin-like (Ig) domains, one single fibronectin type III (FnIII) domain, a MAM (meprin, A5 protein, receptor protein-tyrosine phosphatase μ) domain and a C-terminal containing a cleavage site for GPI (GlycosylPhosphatidylInositol) anchoring to the cell membrane. The presence of multiple Cell Adhesion Molecule-like domains in MDGA1, lead us to hypothesize a functional role related to cellular adhesion for this protein.

Material and methods: For stable transfections MDCK cultured cells were transfected with the pFLAG-MDGA1 construct or the empty pFLAG vector. Transfected cells were selected by growing in DMEM containing 10% fetal calf serum and 500 μg/ml of G418 for 3 weeks. Resistant clones were isolated and MDGA1 expression was analysed by Western blotting. Then MDCK cells expressing MDGA1 were used in cell adhesion assays, invasion assays and cell migration assays.
Results and conclusion: In order to investigate a potential implication of MDGA1 in cellular adhesion, attachment of cultured cells to several ECM proteins such as, collagen type I and IV, fibronectin and laminin was assessed. Moreover, cell invasion was carried out using a modified Boyden chamber assay and finally, MDCK cell motility was assessed using a scratch wound assay. Results seem to indicate that MDGA1 has a functional role related to adhesiveness and motility.

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Sensitivity of single, *in vitro* growing lung cancer cells to gemcitabine measured with synchrotron based fourier transform infrared microspectroscopy

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Background: Chemotherapy has become one of the main treatments for patients with lung cancer. However, it would be ideal to have a tool that could allow clinicians to determine what would be the best combination of chemotherapeutic drugs for each individual patient. To this purpose, we studied whether synchrotron based Fourier Transform Infrared (FTIR) microspectroscopy could become such a tool. This technique uses infrared light that interacts with a sample and measures the vibrational modes of the functional groups of biomolecules present in cells and tissues.
Materials and Methods: Three lung cancer cell lines (A549, CALU-1, and SKMES) were used in this study. Spectra were obtained by seeding 5 x 10^4 lung cancer cells in 100 μL of complete media on aluminium coated slides. After 1 hour incubation at 37ºC and 5% CO_2, gemcitabine at different doses was added to cell cultures. After an overnight incubation, samples were washed three times with 0.9% NaCl. Samples were then kept at −80º before obtaining their synchrotron based micro-FTIR spectra with a Thermo Nicolet Continuum FTIR microscope on beamline 11.1 at the Synchrotron Radiation Source, Daresbury Laboratory, UK. Single cell spectra were recorded with a 10 μm aperture at a resolution of 4 cm^{-1} and with 128 co-additions.
Results: Cell survival decreased proportionally to the addition of gemcitanine. Furthermore, lung cancer cells became rounded and pyknotic following the addition of this drug. This correlated with changes in their micro-FTIR spectra. In fact, the amide I peak at 1645 cm^{-1} (C = O stretching vibrations) shifted to lower wavenumbers following the addition of gemcitabine to *in vitro* growing lung cancer cells. This shift has been associated with cell death.
Conclusion: synchrotron based microscopic FTIR spectroscopy could have a potential as a tool to assess tumour response to chemotherapy at a single cell level.
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OP18/Stathmin expression and phosphorylation influences sarcoma cells invasion and metastasis

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OP18, also known as stathmin, is a cytosolic phosphoprotein firstly identified to be a relay of several intracellular pathways and to be overexpressed in several types of cancer (thus the name of stathmin or OncoProtein 18). Subsequently, it has been demonstrated that OP18 is a microtubules destabilizing protein whose activity is highly regulated by serine phosphorylation. While the role of OP18 expression and phosphorylation has been largely studied in the regulation of mitotic division whether OP18 influences cancer progression is still unclear. Here we report the role of OP18 expression and phosphorylation in sarcoma cells proliferation and motility, following cell-ECM contacts.
We show that OP18 is phosphorylated on three different serine residues (S17, S25 and S38) following adhesion on ECM substrates principally through the activation of the MAPK pathways. In sarcoma cells OP18 expression enhances their motility but is ineffective on their proliferation rate. A mutant defective for adhesion dependent serine 16 phosphorylation is more able in stimulating cell motility through ECM substrates *in vitro*. Expression of the mutant OP18 protein is also able to increases sarcoma cell spreading potential but not local growth *in vivo* in nude mice. Finally, in a panel of human sarcomas OP18 resulted frequently overexpressed respect to the normal counterpart. Interestingly, Op18 is more expressed in recurrent or metastatic respect to primary samples suggesting that it could play a role in sarcoma local or distant dissemination.

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CARF, a collaborator of ARF, regulates p53 functions by affecting MDM2 expression *in vivo*

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CARF, a collaborator of ARF, was first cloned as a novel ARF-binding protein by a yeast interaction screen. It also interacts with p53 directly leading to ARF-independent enhancement of p53 function and in turn undergoes a negative feed back regulation [1,2]. In the present report we describe the *in vivo* and *in vitro* interactions of CARF with p53-anatagonist, MDM2.
Immunostaining and real time visualization by time-lapse video microscope also showed that these two proteins co-localize in the nucleus. It undergoes proteasome mediated degradation via mdm2 mediated ubiquitination. We also found that siRNA mediated silencing of CARF causes up-regulation of MDM2 and down-regulation of p21 and other p53 targeted genes like BAX and PUMA. However, the level of p53 remains unchanged. Simultaneous knockdown of both CARF and MDM2 in U2OS cells failed to down-regulate the p21 expression.
This finding suggests that accumulation of MDM2 in CARF-depleted cells might be responsible for transcriptional inactivation of p53. The data suggests that CARF regulates p53-p21 pathway by more than one way. In addition to its interactions with ARF and p53 proteins, CARF interacts with MDM2 (a terminator of p53 function) and keeps its level in control.

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Transforming growth factor-beta 1 transiently induces Id-1 mRNA through protein kinase C delta and p38 MAP kinase pathway in MDA-MB-231 cells

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Id-1 (Inhibitor of differentiation-1), one of the helix-loop-helix (HLH) proteins, inhibits basic HLH transcription factors from binding to DNA and plays an essential role in the inhibition of differentiation and the cell cycle arrest. To investigate relationship between aggressiveness of breast cancer and Id-1, TGF-β1-dependent regulation of Id-1 has investigated in MDA-MB-231 cells.
The level of Id-1 mRNA is dramatically and transiently increased at 1 hour and disappeared after 3 hours by TGF-β1 in MDA-MB-231 cells (aggressive cells) but was no effect in MCF-7 cells (non-aggressive cells). Id-1 protein was also shown the same result. TGF-β1-induced Id-1 mRNA level was almost reduced by pretreatment of actinomycin-D but enhanced by cycloheximide. In DNase I footprinting analysis, the nuclear factors interacting with the cis-elements were identified in Control and TGF-β1-treated MDA-MB-231: CREB/ATF (−1017) and SBE (−993) binding site, but Egr-1 (−1063) was not detected. The trans-acting factors were bound to each cis-element-specific but quantitative differences were not shown between control and TGF-β1-treated cells. The level of TGF-β1-induced Id-1 mRNA was significantly reduced by Rottlerin (PKCδ-specific inhibitor) and SB 203580 (p38 MAP kinase inhibitor) but was no effect by Gö 6976 (protein kinase Cα, β and μ inhibitor) or PD 98059 (ERK 1/2 inhibitor).
These results suggest that Id-1 may be important to aggressiveness of breast cancer cells and PKCδ and p38 MAP kinase signaling pathway is related to TGF-β1-dependent induction of Id-1 mRNA in MDA-MB-231 cells.