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.

**Reference(s)**

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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).

**Conclusions:** We explored transcriptomic data from three different microenvironments involved in colorectal cancer progression. Levels of stromal chemokines TGFB2 AND NTF3 seems to be important in the progression of colorectal carcinoma and depicted a higher proangiogenic microenvironment in liver metastasis.

### 579 Cytotoxic effects of resveratrol on imatinib sensitive and resistant K562 chronic myeloid leukemia cells

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**Background:** Chronic myelogenous leukemia is a hematological malignancy resulting from translocation between chromosome 9 and 22 that generates BCR/ABL protein. BCR/ABL protein has constitutive tyrosine kinase activity involved in cell growth, differentiation and evasion of apoptosis. Imatinib is the first target specific agent that specifically binds to the ATP binding pocket of the BCR/ABL. However, resistance to imatinib is the major problem of CML patients. Resveratrol is a naturally produced phytoalexin, mostly synthesized in red gapes. It has anti-oxidant, cardioprotective, anti-inflammatory, and anti-tumour activities.
**Aims:** In this study, we aimed to examine apoptotic effects of resveratrol on imatinib sensitive and resistant K562 cells and determine the mechanisms of resveratrol-regulated cell death.
**Methods:** Antiproliferative effects of resveratrol were determined by XTT cell proliferation assay. Apoptotic effects of resveratrol on K562 and K562/IMA-3 cells were determined through changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential (MMP), and apoptosis by caspase-3 colorimetric assay kit, JC-1 MMP kit, and Annexin V-FITC, respectively. On the other hand, expression profiles of BCR/ABL in response to resveratrol were analysed by RT-PCR.
**Results:** IC50 values (drug concentration that inhibit cell proliferation 50% comparing to untreated controls) of resveratrol were calculated as 85 and 122 μM in K562 and K562/IMA-3 cells, respectively. There were 1.91, 7.42 and 14.73-fold increases in loss of MMP in 10, 50, and 100 μM resveratrol applied K562 cells. The same concentrations of resveratrol resulted in 2.21, 3.30, and 7.65-fold increases in loss of MMP in K562/IMA-3 cells. Caspase-3 enzyme activity results showed that there were 1.04, 2.77, 4.8-fold increases in K562 and 1.02, 1.41, 3.46-fold in K562/IMA-3 cells increases in response to the same concentrations of resveratrol, respectively. There were 4 and 3.7-fold increases in apoptotic K562 and K562/IMA-3 cell populations in response to 100 μM resveratrol as compared to their untreated controls. RT-PCR results showed for the first time that resveratrol downregulated expression levels of oncogenic BCR/ABL gene in a dose-dependent manner in both imatinib sensitive and resistant K562 cells.
**Conclusion:** The results of this study may suggest potential use of resveratrol in both responding chronic phase CML and from patients with primary and/or acquired resistance to imatinib.

### 580 Isolation and characterization of a new human lung cancer cell line derived from a metastatic limph node

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**Background:** Lung cancer is currently the most frequently diagnosed cancer and the most common cause of cancer mortality in males (American Cancer Society, 2007).
Low passage tumour cell lines are a powerful model to cancer investigation (Blanco-Aparicio et al, 2005; Moneo et al, 2007). Specifically lung cancer cell lines serve as an invaluable tool for medical science (Gazdar et al, 2010) because the most part of these types of cell lines are very representative of the tumour specimen from which they are derived (Wistuba et al, 1999).We report the isolation and characterization of a new human low passage lung tumour cell culture, from a lung cancer resistant to therapies.
**Material and Methods:** Tumoural fresh sample was obtained from Tumour Bank of San Cecilio Hospital belong to the Tumour Banks of the Andalusia Network, by axilar limph node biopsy from 54-year-old men diagnosed with a metastasic tumour originated by primary lung tumour resistant to chemotherapy and radiotherapy.
Sample was processed and placed in culture with specific media until their generation was achieved. The culture was analysed to determine growth parameters by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and was characterized in various phases of generation for lineage and cycle markers by immunohistochemistry and apoptosis cells rate by ISOL® (in situ oligo ligation). Morever, telomerase activity was quantified by qPCR using telomerase repeat amplification protocol and we performed cytogenetic analysis by G-bands and spectral kariotyping.
**Results:** After incubation for a few weeks, cells grew as firm adherent monolayer with polygonal and epithelial-like morphology, some round and floating cells, and a few multinucleated cells and the doubling time was 48 hours in complete media with 10% FBS. Moreover cells grew in culture media with low serum concentration with 1% FBS.
Immunohistochemistry study showed positive staining for vimentin, cytokeratins, CD44, EGFR, p53, Ki67, Ciclin D1 and B-catenin. Non apoptotic cells were found in any passage. The study for telomerase activity was positive and the cytogenetic in early passage, showed recurrent chromosomal aberrations in lung cancer, with simple and complex structural rearrangements, which was confirmed in later passage.
**Conclusions:** These results indicate that the features of this cell line are closely similar with a lung malignant tumour, and therefore a good tool for cancer investigation.

### 581 Characterization of anaplastic astrocytoma xenografts derived from cancer stem cells in nude mice

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**Background:** The cancer stem cell hypothesis proposes that tumours contain a small subset of cancer cells, the cancer stem cells, which constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumour. The aims of the present study were to investigated the morphology and growing pattern of xenografts derived from cancer stem cells isolated from anaplastic astrocytoma.
**Material and Methods:** Tumours from a patient with grade III glioma (anaplastic astrocytoma) were mechanically and enzymaticaly dissociated and growth in neural stem cell expansion medium to generate neurospheres. The *in vivo* tumourigenic potential of tumour spheres was assayed by intracranial injection of 2 X $10^5$ glioblastoma-derived stem cells into the right striatum of Balb/c nude mice. Tumour growth was monitored *in vivo* by serially sectioning the xenograft brains at two and three months postinjection. Double immunofluorescence for human nestin and PCNA were performed.
**Results:** Astrocytoma xenografts have a small size, indicating a slow growing rate. The xenografts have also a low density cells population which resembles the original tumour-anaplastic astrocytoma. Tumour cells were identified either associated with the white matter tracts of alveus hippocampus or spread in the hippocampus. The shape and size of xenografts varied depending on the mouse. Xenografts obtained from tumour stem cells derived from anaplastic astrocytoma have showed a great number of cells positive for both PCNA and the human nestin. Moreover, distribution of cells positive for human nestin showed diffuse, infiltrative pattern in host brain.
**Conclusions:** Taken together, these results suggest that anaplastic astrocytoma contains cancer stem cells that are able to propagate and can reconstitute the original human tumour *in vivo*.
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### 582 Expression of GSE24.2 prevents DNA damage in X-linked dyskeratosis congenita cells

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Dyskeratosis congenita is rare X-linked recessive disease characterized for presenting mucotutaneus abnormalities, increased cancer susceptibility and bone marrow failure. At the cellular level dyskeratosis congenita cells have short telomeres and premature senescence. We have previously found that an internal domain of dyskerin (GSE24.2) rescues telomerase activity in X-linked dyskeratosis congenita (X-DC) patient cells. Here we have used F9 mouse cell lines expressing the most common mutation found in X-DC patients, A353V, and found that expression of GSE24−2 is able to induce a recovery in telomerase activity in this F9 X-DC mouse model, by increasing the mTR and mTERT RNA levels. SnoRNA levels are not affected. Moreover, a peptide containing the GSE24.2 sequence is also able to directly rescue telomerase activity. F9 X-DC mouse cells show increased DNA damage and expression of GSE24.2 is able to protect from such damage. Further, F9 X-DC mutant cells are more sensitive to DNA damaging agents and GSE24.2 expression rescued both global and telomeric DNA damage. This data indicates that use of GSE24.2 either as a cDNA vector, or as a peptide, could be a suitable approach for therapy of X-DC patients in which by rescuing telomerase activity and DNA damage, may protect from induced cell death or senescence.
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