

this disease is of high priority. ADAMs are a family of metalloproteases 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 changes 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 unknown. 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- α 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 tumorigenic 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, TGFB3, 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).