Conclusions: We show here that flow influences the migration of glioma cells in a 3-D microenvironment. Building upon previous research concerning autologous chemotaxis using CCL21 in carcinoma cells, we show here that there is a similar mechanism in central nervous system tumour migration with the chemokine CXCL12. Thus, we show the first instance of flow directly affecting brain tumour cells and evidence the possibility of autologous chemotaxis in glioma.

440 Molecular basis of the antiproliferative activity of retinoic acid in sensitive breast cancer cells

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Background: Retinoids are used clinically for the treatment of specific malignancies and precancerous conditions, but sensitivity to retinoic acid (RA) is variable in breast tumour cell lines. While it generally correlates with expression of estrogen receptor alpha (ERα)), which regulates expression of the retinoic acid receptor α gene (RARA), some ER-negative lines such as the HER2-amplified SkBr-3 cells are also sensitive to the antiproliferative effects of RA. By identifying RA target genes in ER-positive and ER-negative cells, we seek to better understand the mechanisms underlying transcriptional regulation and growth suppression by retinoids.
Materials and Methods: Primary RAR target genes were identified by gene microarray analysis in ER-positive and ER-negative breast cancer cells pre-treated with the translation inhibitor cycloheximide (CHX) for 1 hour and then with RA for 8 hours. Regulation was confirmed by Q-PCR. Retinoic acid response elements (RAREs) were mapped in RA-regulated genes through bioinformatics. Effects on cell growth of target genes was assessed by colony formation assays and analysis of cell cycle distribution by flow cytometry.
Results: We report here that patterns of gene regulation in both sensitive cell lines are partially overlapping, indicating that part of the antiproliferative effects of RA is independent of estrogen signaling. Differences in gene regulation may result from the different levels of RARα expressed in these cell lines, but can also be partly attributed to major variations in the basal levels of these genes between the two cell lines. In both cell lines, cycloheximide-insensitive upregulated RA target genes are strongly enriched in DR5 response elements. Furthermore, we observe that most genes that were regulated in an antagonistic manner by estrogen and RA were sensitive to cycloheximide for regulation by one of the receptors. Several primary RA target genes common to both cell lines play roles in inhibition of cell cycle progression and survival in ER-negative SkBr-3 cells.
Conclusions: RARs can have antiproliferative effects in ER-negative cells mediated in part by genes similarly regulated in ER-positive cells, suggesting that modulation of the transcriptional effects of estrogens is not the main mechanism of action of RA in breast cancer cell lines.

441 miRNA and cancer stem cell analysis of NSCLC to explain the sensitizing effect of trifluoperazine on cisplatin-induced cell death signaling

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Background: Non small cell lung carcinomas (NSCLC) have a poor outcome and we have reported that failure to activate apoptotic signaling, increased DNA repair capacity as well as increased IGF-1R signaling may be the underlying mechanisms. We previously showed that trifluoperazine (TFP), a small molecule of the phenothiazine class, can sensitize NSCLC to DNA double strand break inducing agents by inhibiting DNA repair, altering cell cycle progression and activating different cell death pathways including apoptosis. Here we aimed to understand if TFP can sensitize for the DNA cross-linking agent cisplatin, one of the mainstay treatment of NSCLC.
Materials and Methods: The NSCLC cells A549 or U1810 were used as model systems. The A549 cells are proficient in p53 whereas U1810 cells do not express p53 due to a silencing mutation. Total RNA was extracted using Trizol Reagent and miRNA and mRNA expression was studied using quantitative real time PCR.
Results: The non-cytotoxic effect of TFP alone on NSCLC was confirmed using clonogenic survival assay. Interestingly, a combination of 10 μM cisplatin and 10 μM TFP was found to inhibit cell growth more efficiently than cisplatin alone in A549 cells (62% vs 76% surviving cells). Similar results were observed in U1810 cells using 5 μM cisplatin and 10 μM TFP. TFP and cisplatin caused increased apoptotic signaling measured as increased caspase-3 activation and affected cisplatin-induced cell cycle pertubations. To identify potential sensitizing mechanisms we next isolated RNA from the surviving clones of untreated, TFP-, cisplatin- or combination-treated cells. At least 500 ng RNA was obtained. Using real time quantitative PCR the expression of stem cell markers was analyzed. Surprisingly Nanog and Sox2 were downregulated in U1810 cells after treatment. Moreover, we analyzed if selected miRNAs expression were different. Our results suggest that miR-1227 is downregulated in both U1810 and A549 cells and miR-214 is downregulated in A549 after treatment. miR-1249 is upregulated in U1810 cells after treatment, with a more pronounced effect by combination treatment.
Conclusion: Our data suggest that TFP has the capacity to sensitize NSCLC to cisplatin. In part this effect may be explained by altered apoptotic signaling propensity. Moreover, our analysis suggests that TFP also has the capacity to alter miRNA expression as a part of the sensitizing mechanism.
L. Lundholm and D. Zong contributed equally to the study.

442 The Abl tyrosine kinase inhibitor Nilotinib inhibits invasive properties of colon cancer cells by targeting the discoidin domain receptor 1

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Background: Tyrosine kinases are frequently deregulated in human cancer and they play important roles in tumourigenesis. They have become promising therapeutic targets and several inhibitors are currently used in the clinic. For example, Nilotinib (Tasigna®), a novel inhibitor of the oncogenic tyrosine kinase Bcr-Abl kinase is used in second line for the treatment of Ph+ Chronic Myeloid Leukemia (CML). Here we addressed whether this drug could also affect neoplastic properties of colon cancer cells (CRC).
Material and Methods: The effect of Nilotinib was assessed on invasive properties of HCT116, SW480, HT29, SW620, Colo205CRC cells both in vitro using Boyden chamber assays and in vivo using intrasplenical xenografts in nude mice.
Results: We found that Nilotinib inhibits the invasion of all CRC cell lines tested. This efficiency was similar to the one observed on the growth of CML (IC50 = 20 nM). Moreover, our results suggest that this effect does not involve any members of the Abl family, but rather the Tyrosine Kinase Receptor DDR1 (Discoidin Domain Receptor 1). DDR1 is the receptor for collagen, one of the main constituent of the extracellular matrix and it has been recently identified as an additional target of Nilotinib (Rix et al, 2007). Accordingly, DDR1 knock-down mimicked the inhibitory effect of Nilotinib. Mutagenesis analyses together with in vivo invasion assay are under way to confirm the role of DDR1 in this transforming process.
Conclusions: Our results suggest that Nilotinib could be of therapeutic value in advanced CRC by targeting the tyrosine kinase DDR1.

443 HMGA2 expression in primary lung carcinomas

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Introduction: Lung cancer affects about 2500 Norwegians annually. Worldwide, lung cancer is the most common cancer both in terms of incidence and mortality. The survival rates for lung cancer remain at about 10%, despite improvements of all treatment modalities the last decades. Increasing the understanding of the biology and detailed molecular characteristics of the tumour is a prerequisite to obtain a better outcome.
One of the proteins overexpressed in lung cancer tumours is the HMGA2 protein. This is a DNA binding and chromatin modifying protein that regulates stem cell renewal, and has been linked to poor outcome in a range of solid cancers. The protein is hardly detectable in normal adult tissue, but abundantly expressed during embryogenesis and in cancers.
The HMGA2 protein seems to play an important role in lung carcinogenesis, and some studies also suggest that HMGA2 could be a rational therapeutic target in lung cancer.
Material and Methods: We have analysed tumour samples from 135 lung cancer patients, of which 68 were adenocarcinomas, 36 squamous cell carcinomas and 31 other histological entities (large cell carcinomas, bronchoalveolar carcinomas and carcinoids).
Immunohistochemistry was performed on tissue micro arrays (TMA) using rabbit anti-HMGA2 (www.biocheckinc.com) and Dako EnVision Flex+ System (K8012). All samples are represented on the TMAs in duplicates. None/weak/moderate nuclear expression was scored as negative (0), less than 10% tumour cells with strong nuclear staining were scored as low expression (1), 10−50% tumour cells with strong nuclear staining were scored as intermediate expression (2) and more than 50% tumour cells with strong nuclear staining were scored as high expression (3). Samples with score 2 and 3 were considered overexpressing HMGA2. Scoring was done blindly with regard to clinico-pathological information.