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.

Results: Totally, 75 (55.6%) samples showed overexpression of the HMGA2 protein. Dividing into histological subtypes, 88.9% of squamous cell carcinomas expressed high levels of HMGA2, while the high expression percentage among adenocarcinomas was 45.6%. For the other histological entities combined, 38.7% showed overexpression. The expression levels were not correlated to overall survival in our study. Progression free survival analyses are ongoing.
Conclusion: In this study, we found a strikingly high percentage of high expression of the HMGA2 protein among squamous cell carcinomas. The expression levels showed no effect on overall survival.

444 Inhibition of Stearoyl-CoA Desaturase induces cell death and activation of AMPK pathway in cancer cells

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Cancer cells exhibit altered glycolysis and lipogenesis metabolisms. Indeed, de novo synthesis of saturated (SFA) and monounsatuarated fatty acids (MUFA) is largely increased in cancer cells. The increase of MUFA, is correlated with a higher level of Stearoyl-CoA desaturase (SCD) activity in variety of cancer tumour. SCD is an endoplasmic reticulum enzyme that introduces a double bond between carbons 9 and 10 of several saturated fatty acids such as stearic acid (converted into oleic acid). De novo MUFA production seems to be required for sustaining proliferation and survival of cancer cells. In contrast, down-regulation of Scd1 leads to proliferation arrest and/or cell death with reduction of lipogenesis witch induces activation of the AMPK pathway, the cellular energy sensor. Its activation has been recently discovered to be involved in cell growth arrest and cell death.
In the present study, we propose to analyse effect of SCD extinction on cell survival and the implication of AMPK pathway in different human cancer cell lines.
Material and Methods: Human adenocarcinoma colic SW480 and osteosarcoma U2OS cells were transfected with siRNA directed against SCD1. Validation of SCD1 extinction was carried out 72h after transfection by HPLC analysis of [^{14}C] stearic acid conversion into [^{14}C] oleic acid in intact cancer cells (desaturation level). We measured protein expression by western-blot, cell viability by Cyquant® and caspase 3 activity by cytometry.
Results: Extinction of SCD1 expression in U2OS and SW480 led to a drastically reduced SCD1 activity with 3% and 4.5% of desaturation level respectively compared to about 35% in the control cells. For U2OS, abolition of SCD1 expression induced a viability decrease (almost 50%) and about 30% of SCD1-depleted cells are positive for active caspase3. We also observed PARP cleavage in depleted SCD1 cells confirming activation of apoptotic pathway. Cell death could not be prevented by addition of 100 μM of oleic acid – a product of SCD1 activity – in depleted SCD1 cells. Then, de novo MUFA synthesis appeared necessary to cancer cell survival. We demonstrated that here the AMPK pathway is activated in depleted SCD1 cells.
Conclusion: MUFA biosynthesis pathway appears as a promising target for cancer therapy since extinction of SCD1, the rate limiting enzyme of MUFA synthesis, leads to cell death of cancer cells.

445 Study of the molecular mechanism of LIF induction by TGF-beta

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Glioblastoma (GBM) is the most common tumour of the adult brain, and it is one of the deadliest tumours, with a median survival of 15 months, despite of the therapies. Because of that, it is of outmost importance to underlie the molecular mechanisms that drive the glioma progression, aggressivity and recurrence, in order to find new treatments.
Recently, our group has demonstrated the importance of the cytokine TGF-beta in glioma progression, showing that those patients with an increased TGF-beta pathway activity have worse prognosis. We are focused in the study of the molecular mechanisms that drive this oncogenic effect of TGF-beta. We want to underlie which are the mediators of this oncogenic effect, and one important mediator is the cytokine LIF (Leukemia Inhibitory Factor). We have demonstrated that the induction of LIF by TGF-beta is crucial for the Glioma Initiating Cells (GICs) self-renewal, enhancing the tumour formation and recurrence. We are especially interested in studying the molecular mechanisms of LIF induction by TGF-beta, as not all the tumours induce LIF in response to TGF-beta.
We studied the LIF promoter region searching for putative transcription factor binding sites, to find possible partners that cooperate with TGF-beta pathway in the LIF induction. We found a putative Runx1 binding site, and we are studying the role of this transcription factor in LIF induction by TGF-beta. We are also interested in its role as an oncogene in GBM.
We are postulating that the Runx1 transcription factor is necessary for LIF induction in response to TGF-beta, so its expression is crucial for tumoural cells in order to increase its self-renewal capacity. We are further studying the role of Runx1 in tumourogenesis and its importance in glioma. Our hope is that, the knowledge about the molecular mechanisms that are involved in the gliomagenesis, will lead us to develop further therapies against this outmost uncurable disease.

446 ETV5 promote epithelial to mesenchymal transition during endometrial carcinoma invasion and is modulated by LPP

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Background: This study aims to characterize the mechanisms of invasion of the endometrial cancer (EC) by focusing on the role of ETV5 and LPP.
Methodology: Differentially expressed proteins were identified by DIGE analysis. In vitro studies were carried out using Hec1a cell line, and its stable clones of GFP-ETV5 upregulation (HGE) and LPP knockdown (sLHGE). We performed immunofluorescence, western blotting and functional assays as videomicroscopy, luciferase and adhesion assays. Chromatin immunoprecipitation was used to identify targets of ETV5. cDNA Microarrays broad our understanding on ETV5 and LPP effects.
Results: Hec1a cell line grows in compact colonies, with well-defined cell-cell contacts. On HGE, cells become disperse, showing a typical mesenchymal phenotype. We report how ETV5 overexpression is able to disrupt cell-cell contacts by decreasing protein and/or mRNA levels of structural proteins, as E-Cadherin at adherens junctions, ZO3 and Claudins at tight junctions and Plakophilin at desmosomes. Furthermore, other proteins localized at contacts like the immunoglobulins and integrins are modified. ETV5 also promote the expression of mesenchymal markers like N-Cadherin or Fibronectin. All these effects are associated with a 2-fold increase rate of migration in HGE. On a first approach to Epithelial-Mesenchymal transition (EMT) we describe how ETV5 is capable to bind ZEB1 promoter, known repressor of E-Cadherin. In addition, we also observe that HGE are more proliferative and more adherent to different matrices than Hec1a. LPP was identified as a protein up-regulated in the invasive stage of EC. We describe how LPP is localized mainly at cell-cell adhesions in Hec1a, and surprisingly, it is relocalized mainly to focal adhesions in HGE. We associate LPP relocalization pattern with ETV5 capability to promote invasion, since transcription based luciferase studies and migration assays on sLHGE revert the increased luciferase expression and increased migratory ability of HGE.
Conclusions: ETV5 overexpression can promote EMT by disrupting cell-cell contacts and increase mesenchymal markers, and also, promote adhesion, increase migration and induce proliferation. Hence, ETV5 would confer to the tumour the invasive capabilities needed to disseminate. In addition, LPP might be a novel coregulatory partner for ETV5 and its relation links LPP to a communication pathway between cell-cell contacts and the nucleus, and implicates LPP in ETV5-associated functions.

447 A story of complexity and discrepancy: CD133 expression and tumourigenicity of colon cancer cell line subpopulations

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Background and Aim: Increasing evidence supports the hypothesis of tumour-initiating/cancer stem cells (TIC/CSC) in solid tumours to relate to poor prognosis and recurrence of disease. The study of cancer subpopulations with exclusive TIC potential is challenging because of the imperfect tools to isolate TIC/CSC, the controversial discussion on the culture methods for their expansion and the divergence in *in vivo* tumourigenicity in diverse animal models. Another subject of fierce debate is the potential of established cell lines to reflect CSC/TIC behavior. CD133 is a biomarker described to identify and/or enrich CSC/TIC from both primary colorectal cancers (CRC) and the established cell line HT29. This could not be verified in other CRC cell lines. Because of the discrepancy, we isolated CD133^{+} and CD133$^{-/low}$ populations from our HT29 cell pool and analyzed *in vitro* survival under defined (treatment) conditions as well as *in vivo* tumourigenicity.
Materials and Methods: CD133^{+}/CD133$^{-/low}$ HT29 and HCT-116 populations were isolated via FACS. 2-D colony formation assays were performed to evaluate cell survival under various milieu conditions (lactate, acidosis) and response to treatment (irradiation, 5-FU, oxaliplatin). 3-D spheroid formation and growth was monitored and *in vivo* tumourigenicity was evaluated in an NMRI (nu/nu) mouse model. CD133 expression was verified by flow cytometry and/or western blotting.