436 Functional analysis of oncogenic YB-1 in the cytoplasm of multiple myeloma reveals that survival is accomplished by stabilizing the mRNA translation of Mcl-1 and TCTP

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The Y-box Binding Protein-1 (YB-1) is an oncogenic transcription/translation factor belonging to the evolutionarily highly conserved family of cold-shock domain proteins. YB-1 binds to DNA as well as RNA and fulfils pleiotropic cellular functions, including the regulation of proteins involved in cellular growth, survival and stress response. In transgenic mice, mammary gland specific over-expression of human YB-1 provokes breast cancer with a 100% penetrance. YB-1 knockout experiments in mice showed that a homozygous deletion is lethal and a heterozygous YB-1 deletion is accompanied with an increased sensitivity to cisplatin and mitomycin C. YB-1 over-expression can be detected in many human malignancies including colorectal carcinoma, prostate cancer, osteosarcoma, breast cancer and multiple myeloma.

Multiple myeloma (MM) is an aggressive B cell neoplasia characterized by the increased proliferation and extended life span of monoclonal plasma cells in the bone marrow. Compared to normal plasma cells YB-1 is strongly expressed in 30−50% of primary MM samples. YB-1 over-expression was seen in a proliferative subset of primary MM cells, which are characterized by Ki67.

Although eukaryotic Y-box binding proteins were originally identified as transcription factors binding to Y-box sequences in promoters of a variety of genes, the protein itself is predominantly expressed in the cytoplasm of primary samples and MM cell lines. Here we present the data of consistent YB-1 bound mRNAs in different MM cell lines and the subsequent functional analysis of promising protein candidates in multiple myeloma.

Using YB-1 immunoprecipitation and gene expression analysis we identified YB-1 bound mRNAs and further characterized them by immunohistochemistry of primary samples, protein knockdown-/over-expression in MM cell lines, western blot analysis, detection of mitochondrial membrane potential and caspase activation.

We found mRNAs coding for anti-apoptotic Mcl-1 and TCTP bound to cytoplasmic YB-1 protein. The shRNA mediated knockdown of YB-1 in different MM cell lines caused a strong decrease of Mcl-1 and TCTP protein levels and induction of apoptosis with subsequent activation of effector caspase 3. Here we present the results how YB-1 enables MM cells to benefit from its anti-apoptotic nature.

In conclusion, YB-1 can function as a RNA-chaperone stabilizing the translation of anti-apoptotic proteins, thus assuring the pro-survival phenotype of MM cells.

437 The role of the SDF1a/CXCR4 axis in invasion of colorectal cancer cells

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The development of distant metastasis is associated with poor outcome in patients with colorectal cancer (CRC). The *stromal cell-derived factor 1α* (SDF1α) and the *CXC chemokine receptor 4* (CXCR4) have pivotal roles in chemotaxis of migrating tumour cells during metastasis. In this regard, there is also evidence for an interaction of SDF1α with *CXC chemokine receptor 7* (CXCR7). Thus, hampering the SDF1α-crosstalk with its receptors bears the excitement of being a promising strategy to suppress metastasis. In this study, we analyzed the invasive behaviour of CXCR4- and/or CXCR7-overexpressing CRC cell lines.

We used lentiviral vectors for overexpression of CXCR4 and/or CXCR7 in the CRC cell lines SW480, SW620 and RKO. Furthermore, knockdown of CXCR4 was achieved using lentiviral shRNA vectors. Endogenous and transgenic CXCR4- and CXCR7-expression was determined by qrtPCR and FACS. Migration towards an SDF1α-gradient was analyzed in chemotaxis and invasion assays. The novel substance Plerixafor™ was used to inhibit CXCR4-mediated migration.

CXCR4-overexpressing cells showed significantly increased invasion towards SDF1α ($p < 0.001$ for SW480, SW620 and RKO), whereas CXCR7-overexpression significantly reduced SDF1α-dependent invasion ($p < 3.17$times;10^{-5} for SW480). Additionally, CXCR4/CXCR7-co-expression resulted in a significant decrease of invasion compared to CXCR4-overexpressing cells ($p < 4.1 \times 10^{-2}$ for SW480). Cells with CXCR4-knockdowns exhibited a significantly lower invasion potential than CXCR4-overexpressing cells ($p < 0.001$ for SW480, SW620 and RKO). Similarly, invasion was significantly reduced by Plerixafor™ pre-treatment of SW480 ($p < 4.4 \times 10^{-7}$) and RKO ($p < 1 \times 10^{-6}$) CXCR4-overexpressing cells. Following Plerixafor™ treatment, the invasive capacity of CXCR4/CXCR7- and CXCR4-overexpressing cells ranged at the same level.

We established CRC cell lines that stably overexpress CXCR4, CXCR7 or both receptors. Chemotaxis and invasion assays revealed that CXCR7 counteracts the invasive potential of CRC cells. In contrast we could significantly demonstrate that CXCR4 is one important factor *in vitro* for CRC cell migration and invasion. *In vivo* assays using the chicken embryo chorioallantoic membrane (CAM) model are ongoing to analyse the participation of CXCR4 in CRC metastasis. Furthermore, our data give first evidence that Plerixafor™ treatment might be a potential strategy to reduce metastasis in CRC patients.

438 Beclin 1 exerts cyto-protective functions independently of autophagy

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Background: Autophagy is a catabolic process during which portions of the cytoplasm are sequestered by double-membraned vesicles and delivered to lysosomes. During the past decade the molecular understanding of this degradative process has been promoted enormously, and currently the role of autophagy in pathogenic events including cancer, neurodegeneration, or infectious diseases is deciphered. Autophagy plays a dual role in tumour promotion and suppression. It appears that the enhancement of autophagy might be useful for the prevention of tumourigenesis and tumour progression, whereas the inhibition of autophagy might support tumour regression. The autophagy-related gene 6 (Atg6/Beclin 1) exerts tumour suppressor effects. Beclin 1 is monoallelically deleted in 40−75% of cases of human breast, ovarian, and prostate cancer.

Material and Methods: Here we describe the generation and characterization of beclin 1−/− DT40 B lymphocytes.

Results: These cells exhibit reduced viability and proliferation rate. LC3 I-to-II conversion appears to be normal upon autophagy induction by thapsigargin. Furthermore, Beclin 1-deficient DT40 cells are sensitized for BCR-induced apoptosis, whereas apoptosis induction by conventional anticancer drugs remained unaltered.

Conclusions: Collectively, our data indicate that Beclin 1 exerts a cyto-protective function in this cellular model system and does not play a role in negative growth control and/or tumour suppression. Furthermore, it appears that Beclin 1 is not absolutely required for autophagic processes.

439 Interstitial flow increases glioma cell migration via a CXCR4/CXCL12 dependent mechanism

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Introduction: Malignant brain tumours have a highly invasive phenotype that makes them difficult to treat with normal therapies. The invasion of gliomas is well documented but still poorly understood and thus difficult to overcome in a clinical setting. Patterns of migration in the brain correlate with cerebrospinal fluid flow patterns. Additionally, it has been well studied that the chemokine CXCL12 has a chemoattractant effect on many gliomas and the degree to which this chemokine attracts glioma cells correlates with malignancy. Here we propose a mechanism for glioma invasion in the brain that is dependent on flow and chemokine gradients. We have previously described a mechanism of autologous chemotaxis by carcinoma cells under interstitial flow conditions via CCR7/CCL21 signaling. We propose that a similar mechanism of flow-induced migration is occurring via the chemokine/receptor pair CXCL12/CXCR4 that is so prevalent in brain tumours.

Materials and Methods: The rat glioblastoma cell line RT2 was used for experiments. Cells were embedded in 3-D matrices consisting of 0.08% hyaluronic acid (Glycosan) and 0.12% collagen I (BD Biosciences) at a total volume of 100 μl in a tissue culture insert (Millipore). Flow was applied by adding culture medium to the top, but not bottom, of the transwell and allowed to flow overnight by a pressure differential. Gels were removed, and inserts imaged and quantified. For live imaging, cells were plated in gels and imaged over 16 hours in an incubated chamber. Cell migration was analyzed using ImageJ tracking programs and MATLAB postprocessing for analysis of migration velocity and distance.

Results: RT2 migration was enhanced 2−3 fold when exposed to flow in a 3-D matrix. Transmigrated cells are expressed as a percent of total cells and this increased from an average of 0.1% of total to 0.3% of total cells ($p < 0.05$). Further, addition of 10 uM of the small molecule CXCR4 inhibitor AMD3100 (Sigma) inhibited cell migration enhancement under flow back to 0.1% migrated cells ($p < 0.05$). Further, exposure of glioma cells to CXCL12 in 3-D gels followed by live imaging showed no alteration in total distance traveled or cell migration velocity at low (10 ng/ml) and high (100 ng/ml) chemokine concentrations, indicating a more complex mechanism than motility enhancement.

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.