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.