liver cancer HepG2 cells, and that the percentage of growth inhibition was 91.1%.
Conclusions: MR-1 is overexpressed in human liver cancer cells and plays an important role in the tumorigenicity. Knockdown of MR-1 blocks the proliferation, adhesion and migration of cancer cells. The data provide evidence for a functional role of MR-1 in the control of MLC2 dependent signaling events as a potential cancer therapeutic target.

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Myofibroblasts and TGF-beta1 induce upregulation of tumoral L1CAM thereby promoting malignant transformation of pancreatic ductal epithelial cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) originating from ductal epithelial cells is characterized by its high malignancy. Since PDAC exhibits strong desmoplastic reaction with stromal pancreatic myofibroblasts (PMFs), PMFs are supposed to drive PDAC tumorigenesis. Previously, we observed high expression of the adhesion molecule L1CAM (CD171) in PDAC cells accounting for chemoresistance. Thus, this study aimed to investigate whether PMFs are involved in the induction of L1CAM in ductal epithelial cells thereby pomoting malignant transformation of these precursor cells and to identify the mechanisms underlying L1CAM induction.
Material and Methods: An in vitro transwell coculture model was employed lasting up to 6 weeks including the immortalized human pancreatic ductal epithelial cell line H6c7 and freshly isolated pancreatic fibroblasts – representative for the main compartment of the tumor stroma. As parameters for apoptosis induction, AnnexinV binding and caspase-3/-7 activity were measured. Cell migration was determined by transmigrationassays in a modified Boyden chamber. Expression of L1CAM and signaling molecules involved in L1CAM induction were detected by western blotting. DNA binding activity of AP-1 subunits was detected by Gelshift- and ChIP assays. Knock down of L1CAM expression for apoptosis and migration assays was performed by siRNA transfection.
Results: When cocultured together with PMFs, L1CAM expression was upregulated in the human pancreatic duct cell line H6c7 in a TGF-b1-dependent fashion accounting for a migratory and chemoresistant phenotype. Accordingly, TGF-b1 treatment of monocultured H6c7 cells increased L1CAM expression thereby enhancing migratory and chemoresistant abilities. Thus, knock down of L1CAM expression reversed the chemoresistant phenotype and diminished cell migration of cocultured and TGF-b1 stimulated H6c7 cells. The TGF-b1- and PMF-induced L1CAM expression was dependent on the activation of JNKs, but not of Smad2/3, a mechanism that was verified in the L1CAM expressing and TGF-b1 responsive PDAC cell lines Colo357 and Panc1. Accordingly, DNA binding activity of the AP-1 subunits c-Fos and c-Jun was detected within the L1CAM promotor of TGF-b1 stimulated H6c7 cells.
Conclusion: These data provide the first explanation how tumoral L1CAM expression is induced and provide new insights into the mechanisms by which PMFs contribute to malignant transformation of pancreatic ductal epithelial cells quite early, thereby promoting tumorigenesis of PDAC. Since L1CAM mediates both chemorestistance and cell migration of PDAC cells, it represents an interesting therapeutic target to overcome chemoresistance and to concomitantly interfere with the process of metastasis.

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Anti-cancer activity of human ribonuclease conjugates with enhanced pharmacokinetic profiles

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The EVade™ Ribonuclease (RNase) technology allows for the transformation of human pancreatic RNase 1 into proteins with anti-cancer activity by substitution of a handful of amino acid residues. The EVade™ RNases selectively cause apoptosis of cancer cells and have demonstrated efficacy in both xenograft and syngeneic models at doses with few to no side effects. An EVade™ RNase called QBI-139 has advanced to a Phase 1 clinical trial. The proteins are small (~15 kDa) and are cleared quickly from the circulation (half life <2 hours in mice, rats and dogs). While the EVade™ Ribonucleases are effective (~70% tumor growth inhibition), improving the pharmacokinetics of therapeutic proteins using polyethylene glycol (PEG) is a common strategy to increase potency. Additional benefits often seen for the PEG-protein conjugates include more convenient dosing and decreased immunogenicity.
The RNases presented were expressed in inclusion bodies in *E. coli* and purified by column chromatography. The RNases were then conjugated to PEGs of varying lengths (5, 12, 20 and 30 kDa). The EVade™ RNase PEG conjugates were tested for their ability to inhibit the growth of human tumors implanted in the flanks of Foxchase nude mice (xenograft models). In addition, the conjugates were tested against murine cancer cells implanted in the flanks of normal mice (syngeneic model). To assess the pharmacokinetics of the EVade™ RNase PEG conjugates, a fluorescence resonance energy transfer (FRET) assay was used to detect the presence of RNase activity in serum of treated animals. Among the EVade™ RNase PEG conjugates tested, QBI-206 has demonstrated up to 20% greater tumor growth inhibition than the first generation drug, QBI-139. This efficacy was achieved at doses using five-fold less RNase per dose with no toxicity. The enzymatic activity of the conjugates demonstrated that systemic exposure (area under the curve) is significantly increased relative to first generation EVade™ RNases, such as QBI-139. Additional studies are ongoing to select an EVade™ RNase PEG conjugate to advance to the clinic. The potency and safety profile of these conjugates provide substantial justification for continued development.

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Molecular pharmacology of benzamide riboside and sodium meta arsenite in chronic myeloid leukemia: a preclinical evaluation

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Background: Chronic myeloid leukemia (CML), a form of leukemia, has serious implications which call for innovative, cheaper but effective modes of treatment urgently.
The **aim** of this study was to evaluate Benzamide Riboside (BR), a new synthetic compound and Sodium meta arsenite (SMA) – previously used in the treatment of acute promyelocytic leukemia, as candidate drugs for CML. While a primary target or sole pathways for SMA induced cytotoxic response on CML has not been reported, BR is known to selectively target and inhibit inosine 5′monophosphate dehydrogenase (IMPDH II) – an isoform present only in highly proliferating cells and requires nicotinamide 5'-mononucleotide adenyl transferase (NMNAT) for its conversion into active form.
Materials: The foremost step was to look for presence of NMNAT, IMPDH in cells in our study. Leukemic cells from CML patients and K562 cells were screened and confirmed for expression of NMNAT and IMPDH by RT-PCR and imunocytochemistry. Subsequently, tumor cells were isolated from peripheral blood of 23 CML patients for exvivo studies. Effect of Glivec®, currently used active agent in the primary treatment of CML, was also studied in parallel.
Methods and Results: BR and SMA induced time dependent and dose dependent cytotoxicity in patient tumor cells as well as in leukemic cell line. Flow cytometric analysis showed that while BR provokes an 'S' phase arrest, SMA arrested the cells in 'G2′ phase. Both BR and SMA caused cell death through selective triggering of apoptosis as apparent from Hoechst, Acridine orange/Ethidium bromide staining (fluorescence microscopy), mitochondrial permeabilization, annexinV assay (flow cytometry), DNA ladder and TUNEL assay. Kinetic spectrofluorimetric studies confirmed involvement of caspases 9 and 3. Expression of apoptosis regulatory molecules – bcl-2, bax, survivin and X-IAP in BR and SMA treated tumor cells were found to be modulated at both RNA (RT-PCR) and protein level (Western blot). Whole genomic microarray analysis on BR and SMA treated K562 revealed up regulation of proapoptotic and cell growth inhibitor genes.
Conclusions: At any given time and dose, the study compounds were more effective than Glivec®, alone or in combination with BR/SMA on leukemic cells. The unifying feature of both BR and SMA seems to be induction of apoptosis. The results, involving primary tumor cells from CML patients, provide preclinical molecular pharmacology model supporting potential utilization of BR and SMA in the management of CML.