in the survival of HCC-BR cells following etoposide treatment as shown by dose response assay, however neither inhibitor had any effect on the survival of etoposide treated HCC-EV cells. In agreement with our dose response assays, treatment with p65siRNA or with BAY-110782 significantly enhanced apoptosis in etoposide treated HCC-BR compared to HCC-EV, as shown by annexin V staining. Therefore we conclude from these studies that NFkB is activated in a BRCA1 dependent manner upon treatment with DNA damaging chemotherapy, and this activation plays a significant role in mediating chemoresistance.

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Inhibition of Stat3 overcomes gefitinib resistance caused by T790M mutation of EGFR tyrosine kinase

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The epidermal growth factor receptor (EGFR) has been a major target of molecular anticancer therapy. Two approaches have been developed, involving monoclonal antibodies and receptor tyrosine kinase inhibitor (TKI), and both have demonstrated benefit in clinical trials. Although anti-EGFR therapies are active in some patients, eventually disease in nearly all patients will become refractory to therapy. Therefore, a better understanding of the mechanisms of resistance to anti-EGFR therapies is critical to further improve the efficacy of anti-EGFR therapy. Mechanisms that mediate resistance to anti-EGFR therapies include autocrine/paracrine production of ligands, secondary mutation, constitutive activation of the downstream pathways, and activation of alternative pathways such as angiogenesis. Here, we show that acquired resistance to gefitnib, an EGFR TKI, might be caused by sustained activation of signal transduction and activator of transcription 3 (Stat3). In the present study, we identified a second mutation of the EGFR gene (T790M) in a patient with gefitinib-sensitive L858R mutation who has eventually progressed after initial response. *In vitro* experiments confirmed that acquired T790M mutation confers resistance to EGFR mutant cells sensitive to EGFR TKI. We found that, among several EGFR downstream molecules, Stat3 signaling was still activated in cells harboring a T790M/L858R mutation with gefitinib treatment. Persistent activation of Stat3 is known to contribute to oncogenesis by several mechanisms, including inhibition of apoptosis, enhancement of cell proliferation, induction of angiogenesis, and suppression of immune responses. Importantly, pharmacologic or genetic inhibition of Stat3 effectively inhibited cell growth in cells with gefitinib-resistant T790M mutation. These suggest that activated Stat3 is a major growth signal derived from T790M mutation and inhibition of Stat3 may overcome gefitinib resistance caused by T790M mutation. Currently, we are searching for novel therapeutic agent which effectively inhibits activated Stat3, and will report final data at the meeting. We propose that combinatorial therapy with EGFR TKI and Stat3 inhibitor may be effective in the aspects of obviating the resistance to EGFR TKI and thus enhancing its therapeutic efficacy.

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Chemoresistance induction by tumor stroma interactions – molecular and epigenetic regulation of caspases

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Background: The failure of all chemotherapeutic strategies is largely based on the profound chemoresistance of pancreatic ductal adenocarcinoma (PDAC) cells either resulting from preexisting intrinsic mechanisms or extrinsicly induced by anti cancer drug treatment itself. In the present study, the impact of the tumor stroma on the induction of chemoresistance of PDAC cells was further elucidated.
Material and Methods: In vitro, a transwell coculture model was employed lasting up to 9 weeks including the chemosensitive human pancreatic carcinoma cell lines T3M4 or PT45-P1 and freshly isolated murine pancreatic fibroblasts – representative for the main compartment of the tumor stroma. Apoptosis was determined by AnnexinV staining. Caspase expression on protein and mRNA level was analysed by westernblot and Realtime-PCR, respectively. In vivo, a SCID mouse model was used inoculating either T3M4 cells alone (mono tumors) or T3M4 cells together with pancreatic fibroblasts (co tumors). Apoptosis and caspase expression in tumor sections was determined by immunohistochemistry.
Results: As we already showed, continuous interaction between tumor and stroma cells results in the induction of chemoresistance, a process involving the proinflammatory mediators nitric oxide (NO) and IL1b. Here, we strengthen these data by the finding that a chemoresistant phenotype is progressively induced during the course of coculture. Cocultured tumor cells showed a continously reduced expression of procaspases-8, -9, -7 and -3 caused by diminished gene transcription. Treatment with the DNA-methylation inhibitor 5-azacytidine significantly enhanced chemosensitivity as well as expression of procaspases in cocultured pancreatic cancer cells. These data indicate that the reduced caspase expression and subsequent chemoresistance in these tumor cells is related to CpG-DNA-hypermethylation, affecting caspase genes themselves (cis) or caspase regulating factors (trans). Using a SCID mouse model, we could show that co tumors were significantly more resistant towards cytostatic drug treatment than mono tumors reflected by reduced tumor sizes and an enhanced number of apoptotic tumor cells. Moreover, caspase expression was clearly diminished in co tumors compared to mono tumors.
Conclusions: These data underscore the role of the tumor stroma in the induction of intrinsic chemoresistance of pancreatic carcinoma and point to the importance of caspases as central target structures in this scenario.

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Drug resistance in highly aggressive acute leukemias is controlled by de novo expressed NG2 proteoglycan acting via modulation of selected transporters

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Background: NG2 surface proteoglycan is emerging as a crucial prognostic factor in certain particularly aggressive acute myeloid (AML) and lymphoid (ALL) leukemias, were it is de novo expressed and intimately associates with MML rearrangements and 4;11 chromosomal translocations, but its transcriptional regulation and biological function in these neoplastic lymphocytes remains unknown.
Materials and Methods: Leukemic model cell lines and patient cells were treated with deacetylating or demethylating agents, or transcriptional repression antagonists. Cells were also transfected with full-length, or deletion constructs of NG2, and assayed for their responsivness to ALL/AML-specific chemiotherapeutic drugs in vitro and in animal models. Drug response was assayed by fluorescence-based cytotoxicity, apoptosis, agonist/antagonist-modulated compound effluxe dynamics, and tumour growth, and was paralleled by DNA microarray, qPCR and proteomic profiling.
Results: We find that NG2 is normally silenced by a p300/CBP/HDAC-dependent repression mechanism involving promoter methylation and global gene profiling identifies sets of genes implicated in this phenomenon. Transient NG2 overexpression in leukemic cells with diverse genotypes confers resistance to different classes of drugs in a cell-cycle-independent manner. Enhanced NG2 expression differentially up-regulates transcription of drug resistance-associated components and modulates the functional activity of MDR1 and MRP1. Leukemic NG2 overexpression is also associated with up-regulation of anti-apoptotic genes and even in this case DNA microarray and global protein expression and phospho-proteomic analyses were employed to dissect the molecular pathways responsible for these changes. The use of deletion constructs identifies an N-terminal Ca^{2+}-binding region of NG2 has critically important in conferring to the cells drug resistance and functional gene screens through lentilviral RNAi libraries are currently employed to assert the hierarchical gene pathways controlled by NG2 expression.
Conclusions: Dismal prognosis of infantile and adult AML and ALL harboring specific chromosomal translocations and MML gene rearrangements may be endowed by the NG2 proteoglycan's ability to perturb the cells' drug resistance and modulate their gene expression patterns. Thus, in these leukemia types, NG2 and its molecular partners are novel therapeutic targets.