854 μM]; [CAIX: 0.0791 μM]), and 3-nitro-N,N-bis(2-chloroethyl)benzenesulfonamide (IC50 [CAI: 317 μM], [CAII: 568 μM], [CAIX: 0.0872 μM]) showed approximately 10,000-fold increase in selectivity towards the CAIX (when compared to CAI and CAII). This enhancement of selectivity may be due to increased binding of the compounds to the active site residues of CAIX (such as Thr193, Thr194 and Zn). The lead compounds are currently being evaluated in combination with cytotoxic anticancer agents in variety of tumour models.

Drug resistance and modifiers

169 POSTER

Sensitisation of neuroblastoma tumours to chemotherapy by use of a novel class of MRP1 small molecule inhibitor

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Neuroblastoma is the most common solid tumour in children under five. Despite recent advances in chemotherapy that have improved the survival rates of other cancers, outcomes for children with this disease remain poor. Aggressive drug refractory tumours often display amplification of the *MYCN* oncogene. Previous results in our laboratory have implicated expression of the Multidrug Resistance-associated Protein (*MRP1*) gene as a powerful prognostic marker in neuroblastoma and also provided evidence that MYCN regulates expression of *MRP1* (*NEJM*, 334:231–8, 1996; *Oncogene*, 23:753−62, 2004; *J Clin Oncol*, 24:1546−53, 2006). To investigate the role of MRP1 *in vivo*, *MYCN* transgenic mice that develop neuroblastoma were crossed with *MRP1* knockout mice to yield tumours either wild type or null for the *MRP1* gene. Engraftment of these tumours into Balb/c *nu*/*nu* mice and treatment with chemotherapy demonstrated that knocking out *MRP1* leads to significantly increased sensitivity to MRP1 substrate drugs. Since clinically relevant modifiers of MRP1 are scarce, we devised a unique cell-based readout system in order to identify small molecule inhibitors of MRP1. The readout system utilized a p53-responsive reporter as a sensor of drug accumulation in cells and was used to screen a 2300 compound small molecule library that had been enriched for modulators of multidrug transporters (*PNAS* 98:14078−83, 2001). Following primary and secondary screening, we have identified several candidate MRP1 inhibitor molecules encompassing four distinct structural classes. These molecules were found to greatly sensitize tumour cells overexpressing MRP1, beyond the levels of known transporter modulators. One compound, 4H10, was investigated *in vivo* for its ability to sensitise tumours to conventional chemotherapy. 4H10, in combination with vincristine (VCR) or etoposide, increased the sensitivity of neuroblastoma tumours to these conventional chemotherapeutic agents in *MYCN* transgenic mice. Similar sensitisation was also observed with human neuroblastoma cells xenografted into nude mice. Initial biodistribution experiments suggest 4H10 increases retention of VCR in the tumour compared to other tissues. This study has therefore identified a novel class of MRP1 small molecule inhibitor that has the potential to be used in the treatment of neuroblastoma.

171 POSTER

Inhibition of the drug-resistant T315I mutant of BCR-Abl

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BCR-Abl is responsible for the proliferation of leukemic cells in the majority of patients with chronic myelogenous leukemia (CML). Imatinib, which inhibits BCR-Abl, is commonly used to treat patients with BCR-Abl positive disease. However imatinib-resistant mutations of BCR-Abl have been widely observed clinically, including the T315I "gate keeper" mutation. It has been shown that this mutant is also resistant to all second-generation BCR-Abl inhibitors, including dasatinib and AMN107.
EXEL-2280 (XL228) is a potent small molecule inhibitor of BCR-Abl which exhibits low nanomolar IC50 values for inhibition of wild-type and imatinib-resistant mutant forms, including T315I and E255K, in biochemical assays. EXEL-2280 also potently inhibits BCR-Abl autophosphorylation and subsequent activation of CrkL and STAT5 in cells expressing wild-type or T315I mutant forms of BCR-Abl. To assess the pharmacodynamic effects of EXEL-2280 on BCR-Abl, K562 cells expressing wild-type BCR-Abl, or BaF3 cells stably transfected with the T315I mutant of BCR-Abl, were implanted into SCID mice to form solid tumors. In both tumor models, a single oral dose of EXEL-2280 inhibited phosphorylation of BCR-Abl and its downstream effectors, CrkL and STAT5, while imatinib only inhibited the wild-type form of BCR-Abl.
These results indicate that EXEL-2280 potently inhibits wild type and drug resistant forms of BCR-Abl in vivo, and provide a rational basis for the clinical development of this agent for the treatment of CML in patients resistant to imatinib and to second-generation inhibitors (i.e. dasatinib) due to the T315I mutation.

172 POSTER

The adhesion molecule L1CAM mediates chemoresistance in pancreatic carcinoma cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant diseases characterized by rapid tumor progression and profound chemoresistance. We recently reported that induction of a chemoresistant phenotype in the PDAC cell line PT45-P1 by long term chemotherapy involves an increased IL1b-dependent secretion of nitric oxide (NO) that in turn accounts for efficient caspase inhibition. In the present study we elucidated the involvement of L1CAM, an adhesion molecule previously found in other tumor diseases, in an IL1b- and NO-dependent chemoresistance.
Material and Methods: L1CAM knock down was performed by using Stealth siRNA. L1CAM expression was analysed by westernblot. Caspase activation and apoptosis induction was determined by a luminiscent caspase-3/-7 assay. iNOS expression was analysed by Real-time PCR and NO secretion in cell culture supernatants was determined by NO assay based on Griess reaction. L1CAM expression in human pancreatic carcinoma specimens was analysed by immunohistochemistry.
Results: Chemoresistant PT45-P1res cells, but not chemosensitive parental PT45-P1 cells, express high levels of L1CAM in an ILb-dependent fashion. L1CAM knock-down in PT45-P1res cells efficiently reduced iNOS expression and NO secretion and led to an increase of anti cancer-drug induced caspase activation, an effect reversed by the NO donor SNAP. Interestingly, L1CAM ectodomain shedding, i.e. by ADAM10, as reported for other L1CAM related activities, seems to be dispensable for anti-apoptotic protection by L1CAM. Neither the shedded L1CAM ectodomain was detected in chemoresistant L1CAM expressing PT45-P1 cells nor did the administration of various metalloproteinase inhibitors affect L1CAM-dependent chemoresistance. Immunohistochemical analysis revealed L1CAM expression in pancreatic cancer specimens supporting a potential role of L1CAM in the malignancy of this tumor. These findings substantiate our understanding of the molecular mechanisms leading to chemoresistance in PDAC cells and indicate for the first time an important role of L1CAM in this scenario. Furthermore, L1CAM might also be of importance for invasion and metastasis of pancreatic carcinoma cells, a role which has to be defined yet. Taking all these findings into account, L1CAM represents an interesting therapeutic target to overcome chemoresistance and to concomitantly interfere with the process of metastasis.

173 POSTER

NFkB is a critical mediator of BRCA1 induced chemoresistance

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Our group has previously reported using multiple cell line model systems that BRCA1 mediates resistance to a range of DNA damaging agents including etoposide, cisplatin and bleomycin. In agreement with these preclinical models a number of clinical retrospective studies have shown that BRCA1 mutant tumours are more sensitive to DNA damaging chemotherapy compared to tumours from matched non-carrier patients, and that BRCA1 mutation carriers have a worse overall survival if they do not receive adjuvant chemotherapy. NFκB is a well-documented mediator of chemoresistance in response to DNA damaging agents. The aim of this study is to evaluate the role of NFkB in mediating BRCA1 dependent chemoresistance. We examined the activation of NFκB by electromobility shift assay (EMSA) in BRCA1 deficient HCC-1937 cells that had been reconstituted with BRCA1 (HCC-BR) or empty vector (HCC-EV), following treatment with etoposide. There was a clear activation of NFκB in HCC-BR cells treated with etoposide compared to HCC-EV cells. Similarly we demonstrated that siRNA mediated inhibition of endogenous BRCA1 in T47D cells abrogated etoposide induced activation of NFκB as shown by EMSA and luciferase assays, where luciferase was placed under the control of an NFκB promoter. To investigate the consequences of NFκB activation on cell survival, NFκB was inhibited using either siRNA against the p65 subunit of NFκB, or with the chemical inhibitor BAY-7082. Treatment with either of these inhibitors caused a significant reduction

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.

174 POSTER

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.

175 POSTER

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.

176 POSTER

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.