

854 μM]; [CAIX: 0.0791 μM]), and 3-nitro-N,N-bis(2-chloroethyl)benzene-sulfonamide (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 *nulnu* 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 sensitize 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 western blot. 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. NFkB 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 NFkB by electrophoretic 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 NFkB 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 NFkB as shown by EMSA and luciferase assays, where luciferase was placed under the control of an NFkB promoter. To investigate the consequences of NFkB activation on cell survival, NFkB was inhibited using either siRNA against the p65 subunit of NFkB, or with the chemical inhibitor BAY-7082. Treatment with either of these inhibitors caused a significant reduction