photo-affinity linker and the cell lysates that overexressed DsRed or DsRed-fused Pirin were used.
Results: We identified a small-molecule that binds to Pirin by using the chemical array screening method. The K_d value of the small-molecule to Pirin was 614 nM by isothermal titration calorimetry experiments. The pull-down assay showed that the small-molecule inhibited the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. To get a insights into the effect of the small-moleclue against cells, we examined the cytotoxic activity against a number of different human cancer cell lines. As a result, the small-molecule did not show potent cytotoxic activity. In contrast, the inhibition of migration in melanoma cells was observed in the dose-dependent treatments of the small-molecule, but not the treatment of the negative control that does not bind to Pirin. We found that the treatment with the cultured tumor cells with the small-molecule or Pirin-targeted siRNA resulted in suppression of migration, but not cell proliferation.
Conclusions: We have discovered the first inhibitor of Pirin, functions in cellular system is unclear. The inhibitor disrupted the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. By use the inhibitor and siRNA, it is suggested that Pirin regulates migration of the tumor cells, and the inhibitor of Pirin might be a new chemotherapeutic potential for invasive tumors.

356 POSTER

An innovative platform technology accelerates drug screening

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Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) are biophysical techniques that enable precise and sensitive analysis of molecular interactions in solution. FCCS allows analysis of reactions in realtime at nanomolar concentrations and can be applied to the monitoring of binding events in cellular lysates and living cells.
FCS and FCCS are single molecule sensitive fluctuation analysis methods; in contrast to other fluorescence techniques, the parameter of primary interest is not the emission intensity itself, but rather spontaneous intensity fluctuations caused by minute deviations from thermal equilibrium. Consequently, FCS/FCCS can only function properly if concentrations and observation volumes are reduced such that only few molecules are simultaneously detected. For this, the observation volume is defined by strongly focused, overlapping excitation laser beams and a confocally arranged optical setup. Fluorescent molecules entering and leaving this open, illuminated spot of below 10^{-15} liters volume by diffusion emit photons, as long as they dwell in the observation focus. In combination with single photon sensitive detectors the photon count trace is recorded and subjected to a mathematical operation, the auto- and cross correlation analysis, to extract information on particle number, brightness, size and interactions.
The authors will demonstrate the application of FCCS addressing several relevant cancer targets and reference inhibitors. Esp. the quantitative determination of binding constants within cellular lysates enables the efficient and sensible characterization of kinase inhibitors with long residence time.

357 POSTER

Antitumor triazoloacridinone C-1305 as a potent FLT3 tyrosine kinase inhibitor in human acute myeloid leukemia (AML) cells

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Background: An internal tandem duplication (ITD) in the juxtamembrane domain of Fms-like tyrosine kinase 3 (FLT3) is the most common molecular defect associated with acute myeloid leukemia (AML) and predicts for poor prognosis. This mutation causes constitutive activation of the FLT3 receptor in the absence of natural FLT3 ligand (FL). Here, we investigated whether antitumor triazoloacridinone C-1305, selected for extended preclinical trials, exhibits inhibitory activity toward FLT3 kinase.
Materials and Methods: C-1305 activity was evaluated in human leukemia cell lines, MV4;11 (FLT3 ITD) and RS4;11 (FLT3 WT). The inhibitory effect of C-1305 on FLT3 receptor activation was determined by analysis of protein phosphorylation by ELISA. Flow cytometry for annexin V/PI assay, caspase-3 activation and sub-G1 DNA fraction was applied to identify apoptosis. DAPI staining was used to analyse cellular morphology.
Results: Ligand-independent phosphorylation of FLT3 in MV4;11 cells was inhibited by C-1305 with an IC_{50} of approximately 7 μM. Treatment of MV4;11 cells with the drug for 72 h suppressed cell proliferation with EC_{50} concentration equal to 0.2 μM. To determine whether blocking of FLT3 activity modulates cellular response upon C-1305 treatment, MV-4;11 cells were exposed to various drug concentrations. C-1305 was found to exerts its cytotoxic effect by the induction of apoptosis as evidenced by chromatin condensation, phosphatydilserine externalization, caspase-3 activation and presence of sub-G1 fraction. In all tests, the number of apoptotic cells increased after treatment with C-1305 in a time- and dose-dependent manner and reached about 80% after 72 h of drug exposure. Importantly, apoptotic cell death was observed at concentration of drug required to block FLT3 phosphorylation, suggesting that inhibition of FLT3 kinase by C-1305 may account for its cytotoxic activity in MV4;11 cells. Since majority of AML harbor WT-FLT3, we further tested the effect of C-1305 on RS4;11 cells. Significantly higher concentration of the drug (EC_{50} 1,8 μM) was required to inhibit the growth of RS4;11 cells, compared to MV4;11, suggesting potent selectivity of C-1305 towards FLT3 ITD cells.
Conclusions: Our results indicate that C-1305 shows high potency and selectivity toward acute leukemia cells harboring FLT3 ITD activating mutations as evidenced by inhibition of cellular proliferation, induction of apoptosis and inhibition of FLT3 autophosphorylation in these cells.

358 POSTER

Study of cetuximab and panitumumab activity in an 80 cell line panel to evaluate specificity of small molecule EGFR inhibitors

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Developed and broadly applied in the Developmental Therapeutics Program of NCI/NIH, multiple cell line panels became essential platforms for anticancer drug discovery and development efforts. An activity profile of an anticancer agent in a large cell line panel can lead to hypotheses about its mode of action when compared with reference agents. Antibodies with naturally inherited specificity of action could be better reference molecules, but they are hardly ever used for these purposes. This can be explained by a number of factors. First, antibody responses cannot be described by a common sigmoid dose response curve. Therefore, statistical correlation methods cannot be applied for comparison as performed with small molecules. Secondly, antibody activity *in vivo* is linked to secondary responses, e.g. Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), which partially explain only minor effects of antibodies on the growth of tumor cell lines *in vitro*.
We will present an approach to implementing activity profiles of antibodies for studying the mode of action of small molecules. We tested cetuximab and panitumumab in a panel of 80 cell lines *in vitro*. Both agents were applied to cells in a range of 0.01 μg/ml to 100 μg/ml for 72 hours, and their activity was measured by the sulforhodamine B assay. Based on the dose response curve we selected three different groups of cell lines. The first group included cell lines, e.g.NCIH292, most probably dependent on EGFR activation, and very sensitive (already at 0.1 μg/ml) to the antibody action. The second group comprised cell lines, e.g. A431, that were less sensitive to the antibodies. Resistant cell lines were combined into the third group. Activity profiles of small molecules were used to match activity patterns of these three groups. Among more than 300 reference anticancer agents, the strongest similarity was observed for EGFR inhibitors, e.g. lapatinib or erlotinib. Less, but still significant similarity was observed for agents known to be involved in the EGFR pathway: some Akt, MEK and PI3K inhibitors. These results indicate that antibodies can be applied to study small molecule's mode of action. In addition, we observed that the molecular profile of cell lines sensitive to the antibodies correlated well with the known clinical outcome for these antibodies. This fact suggests that larger sets of cell line models should be considered for pre-clinical evaluation of antibodies independently from an ADCC study.

359 POSTER

Effect of the TGF-beta 2 specific antisense oligodeoxynucleotide trabedersen on TGF-beta 2 and -beta 1 expression in human glioma cells: Cross-regulatory loops regulate TGF-beta isoform expression

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Background: Transforming growth factor-beta (TGF-beta) plays a key role in cancer progression, by regulating crucial cancer mechanisms. Three mammalian isoforms, i.e. TGF-beta 1, 2, and 3, have been isolated. TGF-beta 2 plays a particular role in several cancers, and our newly developed therapeutic approach based on the specific inhibition of TGF-beta 2 synthesis by the antisense oligodeoxynucleotide trabedersen (AP 12009) has been successfully tested in clinical trials in patients with advanced stage glioma, pancreatic cancer or malignant melanoma.
Apart from TGF-beta 2, most tumors also express considerable amounts of TGF-beta 1. Since auto-induction and cross-regulation of different TGF-beta isoforms has been reported in some tissues, we were investigating, if this phenomenon is also present in human tumor cells and

particularly, if inhibition of TGF-beta 2 by trabedersen also affects TGF-beta 1 levels.
Material and Methods: The human glioma cell line A-172 was used. Cells were treated with trabedersen or recombinant human TGF-beta 2 (rhTGF-beta 2). Cellular proliferation was assessed via DNA quantification. TGF-beta protein levels in cell culture supernatants were determined by ELISA, mRNA was quantified by qRT-PCR.
Results: While in cell culture medium containing serum, trabedersen potently inhibited TGF-beta 2 expression and barely affected TGF-beta 1 expression, a strong inhibition of both isoforms was observed under serum-free conditions. Serum contains considerable amounts of TGF-beta 1 and 2, which may affect TGF-beta expression by auto- and cross-regulatory loops. Treatment of cells with rhTGF-beta 2 strongly induced expression of TGF-beta 1 on the mRNA as well as on the protein level. The inhibitory effects of trabedersen on TGF-beta 1 expression could be reversed by addition of rhTGF-beta 2. Cellular proliferation of A-172 cells was not affected by trabedersen or rhTGF-beta 2, which might be explained by the fact that growth of A-172 cells is not affected by TGF-beta.
Conclusions: We have demonstrated that by cross-regulatory loops TGF-beta 2 induces the expression of TGF-beta 1 in human glioma cells. Specific inhibition of TGF-beta 2 expression by trabedersen interrupts this cross-regulatory loop and additionally down-regulates TGF-beta 1. This combined inhibition of different TGF-beta isoforms is assumed to contribute to the potency of trabedersen in tumors expressing several TGF-beta isoforms.

360 POSTER

BIO, the GSK3 beta blocker, is a potent inhibitor of cell proliferation and inducer of cell death of cervical carcinoma and rhabdomyosarcoma tumor cells

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Introduction: Since tumor disease is still an very important clinical problem new therapeutic strategies are needed. Cervical carcinoma (CC) is one of the major causes of death among women suffering from tumor. The highest mortality is observed in the group of patients with late diagnosis and with a metastatic disease. Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Also in this case the mortality is very high in patients with metastatic disease. The 5 year survival years in this group of patients is only about 20%.
Objectives: We have studied the influence of GSK3b inhibitor BIO on cervical carcinoma and rhabdomyosarcoma cell lines proliferation and survival.
Materials and Methods: CC cell lines (HeLa and HTB35) and RMS cell lines (RH30 and SMS) were used as a models. The influence of BIO on cell proliferation was studied using cell count assay and MTT assay. To check the influence of BIO on cell survival we used Hoechst 33342 staining and Annexin V and Caspase 3 staining. We also studied the influence of BIO on normal cells (MSC, HUVEC and myoblasts) and nonmalignant satellite cell lines (C2C12). The expression and activation of GSK3b was studied using western blot.
Results: We used three doses of BIO − 1, 5 and 10 uM. The lowest concentration was not able to inhibit proliferation of tumor cells or to induce apoptosis. Moreover, at this concentration in some instances proliferation was even increased. At higher doses the strong inhibitory effect on cell proliferation and survival was observed. We used two different rhabdomyosarcoma cell lines reflecting two RMS subtype. Interestingly, the alveolar subtype which is recognized as more malignant and highly metastatic was significantly more sensitive to the BIO. This phenomenon could be related to the different expression of GSK3b in this subtype in comparison to the embrional one (SMS). The cervical carcinoma cells were highly sensitive to the BIO inhibition as well. After 96 hours of incubation at the highest concentration of BIO more than 95% was inhibited in proliferation assay. At the same time profound cell death was observed. 5−10 uM BIO exerts massive cell death in HUVECs. On the other hand, mesenchymal stem cells and muscle cells were much more resistant to the action of BIO.
Summary: In the war with tumor new therapeutic strategies are still needed. In this study we showed for the first time that blocking of GSK3 function by specific small molecule inhibitors is able to block proliferation of cervical carcinoma and rhabdomyosarcoma cells and decrease their survival.

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HGF regulates the activity of GSK3 in rhabdomyosarcoma cells

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma among children. Two major subtypes are recognized based on morphological and molecular features: alveolar (ARMS) and embryonal (ERMS). There are major differences between AMRS and EMRS e.g. presence of fusion proteins and higher rate of metastasis into distant organs like lungs and bone marrow in case of ARMS. Epithelial–mesenchymal-transition (EMT), is a phenomenon whereby epithelial cells temporarily or permanently acquire mesenchymal phenotype. EMT has been shown to play a key role during embryogenesis and wound healing. Latest reports have shown that EMT plays a crucial role in the development and progression of various tumors.
Aim: Dissecting the role of HGF in regulation of GSK3 activity and EMT related genes.
Materials and Methods: Cell lines used in experiments: RH30 (ARMS), SMS-CTR (ERMS). Real time RT-PCR and western blotting to evaluate gene expression and activation of various intracellular signaling pathways, respectively. Evaluation of protein activation in RMS cell lines was performed after stimulation with HGF.
Results: We observed the phosphorylation of GSK3b on serine 9 after HGF stimulation. To dissect the intracellular pathways responsible for GSK3b phosphorylation several inhibitors such as PI3K inhibitor − Ly294002, MEK inhibitor − U0126 and MET phosphorylation inhibitor were used. Use of both MET and PI3K inhibitors completely attenuated phosphorylation of GSK3b in HGF stimulated RMS cells. We observed also the accumulation of beta-catenin and Snail1 in the nucleus of RMS cells stimulated with HGF. This effect was father augmented when HGF was used together with BIO, small molecular inhibitor of GSK3b. When we study the expression of genes regulated by GSK3b, Snail1 and beta-catenin we noticed downregulation of E-cadherine expression and upregulation of cyclin D1 in cells stimulated with HGF.
Conclusion and future directions: Based on our data we can postulate that stimulation of GSK3b phosphorylation by HGF leads to stabilization and nuclear translocation of EMT activating proteins Snail1 and beta-catenin. This action subsequently causes the downregulation of E-cadherin and upregulation of cyclin D1. Finally, we think that GSK3b could be used as the new therapeutic target to block invasion and metastasis of RMS.
Future experiments will include (i) silencing of *GSK3b* (by viral transduction with shRNA), (ii) establishing the level of GSK3b expression at protein level in different RMS cell lines (by Western Blot) and RMS patients (by immunohistochemistry).

Molecular-targeted therapies − clinical trials

362 POSTER

First in human trial of a poly(ADP)-ribose polymerase (PARP) inhibitor MK-4827 in advanced cancer patients (p) with antitumor activity in BRCA-deficient and sporadic ovarian cancers

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Background: MK-4827 is a potent, selective, PARP1/2 inhibitor with IC_{50} of 3.8 nM. It induces selective synthetic lethality in homologous recombination (HR) repair deficient tumors with BRCA1/2 loss and in tumor cell lines with non-BRCA-related HR defects, supporting clinical utility in sporadic tumors.
Methods: MK4827 was administered orally once daily in cohorts of 3−6 p, enriched for BRCA-deficient and sporadic cancers associated with HR repair defects. Dose escalation was guided by toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) data. Permission was obtained from the appropriate regulatory authorities and properly informed consent given.
Results: 59 p (M13, F46; median age 56 years; 23 BRCA-mutation carriers) were treated at 10 dose levels [30 mg (n = 6), 40 mg (n = 3), 60 mg (n = 7), 80 mg (n = 6), 110 mg (n = 5), 150 mg (n = 6), 210 mg (n = 6), 290 mg (n = 5), 300 mg (n = 9), 400 mg (n = 6)] on days 1−21 of a 28 day cycle (C) in C1, followed by continuous dosing. Prior systemic treatments were 1−2 (n = 5p), ⩾3 (n = 11 p), and ⩾4 (n = 40 p). Overall, dose-limiting toxicity was observed in 4 p: grade (G) 3 fatigue in 1/6 p at 30 mg, reversible G3