in the mice bearing HCC827 xenograft tumors, EZN-3920 administered intravenously in saline (q3dx5) was shown to be highly effective at inhibiting tumor growth as well as down-modulate HER3 and the PI3K/AKT signaling pathway in the tumors. EZN-3920 is currently being evaluated in a xenograft model of the gefitinib-resistant cells.
We conclude the following: (1) down-regulation of HER3 by an LNA antisense molecule is an effective method to inhibit tumor cell growth both in vitro and in vivo, (2) gefitinib hypersensitivity may indicate that cells are dependent on HER3 and will be inhibited by HER3 antisense molecules, (3) sustained activation of HER3 in the presence of down-regulation of phospho−EGFR may be just as important as HER3 hyperactivation in gefitinib-resistant cells. Furthermore, pharmacological manipulation to down-regulate HER3 by EZN-3920 could prove to be a translational approach to controlling HER3-mediated tumor growth in cancer patients.

308 POSTER

Internalization systems of EGFR could affect the efficacy of gefitinib in NSCLCs with wild-type EGFR

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Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have had a significant impact on non-small-cell lung cancer (NSCLC) outcomes. Recent studies have established that most EGFR mutant non-NSCLCs are sensitive to EGFR TKIs, but many EGFR wild type NSCLCs are resistant to TKIs. Moreover, although most of the functions of EGFR have been discussed on its kinase activity, EGFR also takes part in complex set of interactions in the cytosol or even in the nucleus, implicating its important role in proliferation and survival of cancer cells. However, intracellular change of EGFR that lead to resistance to therapies has not been fully understood. Therefore, we have investigated whether alternative resistance mechanism to gefitinib is existed in NSCLC cells with wild-type EGFR. To confirm whether inhibition of EGFR has any effect on cell growth, we evaluated growth inhibitory effects of gefitinib in NSCLC cells with wild-type EGFR (H358, H1299 and Calu-1) using both a tetrazolium (MTT) colorimetric assay and direct cell counting. H358 cells were more sensitive to gefitinib than H1299 and Calu-1 cells. In addition, gefitinib had a striking effect on cellular morphology of H358 cells but not of H1299 and Calu-1 cells. To study that these differences between the cell lines is associated with significant change in metabolism of EGFR, we confirmed the activation status of EGFR and the downstream mediators of EGFR using Western blot assay. However, we did not find significant differences on the activity status of the EGFR associated proteins between these lung cancer cells. Subsequently, we determined whether intracellular changes of EGFR show different patterns after gefitinib treatment in these cells using flow cytometry and immunofluorescence microscopy. EGFR cellular internalization in H358 cells was inhibited by gefitinib but not H1299 and Calu-1 cells. These results suggest that the internalization systems of EGFR could affect the efficacy of gefitinib in NSCLCs with wild-type EGFR.
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309 POSTER

Potentiating the anti-tumor efficacy of molecular targeted therapy for hepatocellular carcinoma by inhibiting the insulin-like growth factor signaling pathway

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Background: Insulin-like growth factor (IGF) signaling pathway has been demonstrated an important regulatory mechanism of tumorigenesis and drug resistance in many cancers. Previous studies have shown that inhibition of IGF signaling may induce apoptosis and reverse resistance to cytotoxic agents in hepatocellular carcinoma (HCC) cells. The present study explored the potential synergistic effects between IGF receptor inhibition and other molecular targeted agents in HCC cells.
Material and Methods: HCC cell lines tested included Hep3B, PLC5, and SK-hpe1. The molecular targeted agents tested included sorafenib, sunitinib, erlotinib, and the IGF receptor kinase inhibitor NVP-AEW541 (Novartis). The potential synergistic antitumor effects were tested by MTT assay and median dose effect analysis in vitro and by xenograft models in vivo. Apoptosis was analyzed by measuring the subG1 fraction and annexin V staining using flow cytometry. The activity of pertinent signaling pathways and expression of apoptosis-related proteins were measured by Western blotting.
Results: IGF can activate IGF receptor and downstream AKT and ERK signaling activities in all the HCC cells tested, but the growth-stimulating effect of IGF was most prominent in Hep3B cells. NVP-AEW541 can abrogate IGF-induced activation of IGF, AKT, and ERK signaling in HCC cells. Synergistic growth-inhibitory and apoptosis-inducing effects in HCC cells were found when NVP-AEW541 was combined with sunitinib or erlotinib but not with sorafenib. These synergistic effects are independent of inhibition of IGF receptor, AKT, and ERK activities by NVP-AEW541. The synergistic anti-tumor effects between sunitinib and NVP-AEW541 were confirmed in vivo by xenograft models.
Conclusion: The apoptosis-potentiating effects of IGF signaling blockade for HCC may be drug-specific. Combination therapy of IGF receptor inhibitors with other molecular targeted agents may improve the therapeutic efficacy in HCC.
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310 POSTER

Acquired resistance to HSP90 inhibitor and cancer progression

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Heat shock protein 90 (HSP90) is a molecular chaperone required for the stability and function of many proteins. The chaperoning of mutated and over-expressed oncoproteins by HSP90 enhances survival, growth and invasive potential of cancer cells. Many HSP90 inhibitors, including the benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin (17-AAG), are currently in clinical evaluation. However the mechanisms and implications of acquired resistance to this class of drug remain largely unexplored.
We have generated isogenic human breast cancer cell lines that are resistant to 17-AAG by continued culturing in the compound. Growth inhibition assay was performed to assess the sensitivities of cells to HSP90 inhibitors. Gene expression profiling, qRT-PCR and western blot analysis were performed on the parental and resistant cells. *In vitro* cell biology were assessed using proliferation, migration and wound healing assays. Intracardiac injection of parental and resistant cells was done in nude mice to assess the metastatic propensity of the cells *in vivo*.
High levels of resistance were maintained in the 17-AAG resistant cells after cessation of treatment. Cross resistance to other ansamycin benzoquinones such as geldanamycin and 17-DMAG were observed, as well as to the structurally unrelated compounds radicicol and CCT018159. The resistant cells demonstrated a significant increase in chemotactic migration and accelerated wound closure *in vitro*. *In vivo* study using xenograft mouse model showed decreased metastasis of the resistant cells to soft organs following intracardiac inoculation. However, x-ray analysis showed enhanced bone lesions in mice inoculated with resistant cells. Gene array and western blot analyses showed that bone marrow stromal cell antigen 2 (BST2) is elevated significantly in the resistant cells. BST2 has been previously linked to increased bone metastasis in breast cancer cells. In addition, IGF-I receptor (IGF-1R), Focal adhesion kinase (FAK), and activated AKT are also upregulated significantly.
These results indicate that acquired resistance to HSP90 inhibition is accompanied by changes in cancer cell biology which potentially leads to increase in bone metastasis.

311 POSTER

MAGEA tumour antigens mediate platinum cytotoxicity in NSCLC

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Background: Resistance to platinum-based chemotherapy is a major problem in the treatment of non-small cell lung cancer (NSCLC) patients. We generated a panel of platinum-resistant NSCLC cell lines to interrogate mechanisms of resistance.
Materials and Methods: We developed platinum-resistant A549 cells by exposure to incrementally increasing concentrations of cisplatin-, carboplatin- or oxaliplatin and assessed drug cytotoxicity by MTT. Details of the NSCLC patient cohort have been previously published.[1] Gene expression in patients[1] and cell lines was measured on Affymetrix

HGU133A microarrays and confirmed by real-time qPCR and western analysis. A549 stable transfectants were generated by subcloning MGC IMAGE clones into pcDNA3.2/V5-DEST prior to liposome mediated transfection and G418 selection.
Results: Cisplatin- and carboplatin-resistant cells were equally cross-resistant to either drug, but interestingly twice as sensitive to oxaliplatin compared to the parental line. Oxaliplatin-resistant cells had moderate cross resistance. Thus cross resistance patterns reflect clinical observations of efficacy. Microarray analysis identified transcripts significantly correlated with resistance of NSCLC cells to all platinum drugs. Two melanoma antigens, MAGEA3 and MAGEA6, members of the cancer/testis antigen (CTA) family, were of particular interest due to their reported tumour specific expression in adult somatic tissues, with frequent expression in lung cancers and a proposed role in lung tumorigenesis. Furthermore, MAGEA3 and MAGEA6 are the most commonly expressed CTA in lung cancers. MAGEA3 and MAGEA6 were strongly expressed in the sensitive parental line, but with minimal or undetectable mRNA or protein expression in the platinum-resistant lines. Furthermore, MAGEA3 and MAGEA6 were only strongly expressed in NSCLC tumours from patients who responded to platinum-based chemotherapy, with none of the refractory patients expressing significant levels. Functional studies demonstrated that exogenous expression of MAGEA3 or MAGEA6 restored sensitivity of resistant NSCLC cells to platinum drugs.
Conclusions: Loss of MAGEA3 and MAGEA6 is a mechanism of platinum resistance in NSCLC. In light of the ongoing clinical trials of anti-MAGEA3 immunotherapy in NSCLC, further investigation of the MAGEA antigens is necessary to optimise scheduling of immuno- and cytotoxic therapy in NSCLC patients. Furthermore, restoration of MAGEA3 or MAGEA6 expression or signalling may restore sensitivity of refractory NSCL tumours to chemotherapy.

References

[1] Petty R, Kerr K, Murray G, Nicolson M, Rooney P, Bissett D, Collie-Duguid ESR. J Clin Oncol 2006: 24, 1729–1744.

312 POSTER

MET and KRAS gene amplification mediates acquired resistance to MET tyrosine kinase inhibitors

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Background: The recent introduction in cancer therapy of several selective tyrosine kinase inhibitors has had a dramatic effect in oncology. However, after the first excitement following the initial results, the problem of acquired drug resistance has become more and more important and still represents a crucial limitation. One of the challenges related to targeted therapies is, therefore, to predict mechanisms that could cause resistance to the treatment and to find ways to circumvent these hurdles. The role of MET − the receptor for Hepatocyte Growth Factor − in human tumors, established by genetic and clinical data, led to the development of small-molecule inhibitors that are in clinical trials. So far, it is not possible to draw any conclusion about their therapeutic efficacy and the emergence of resistance to treatment, a problem that has been often observed with other kinase inhibitors. Studies aimed at investigating the molecular mechanisms responsible for resistance to therapies targeting other kinases have underscored the validity of preclinical models to identify physiologically relevant mechanisms of resistance.
Materials and Methods: To predict mechanisms of acquired resistance, we generated resistant cells by treating MET-addicted cells with increasing concentrations of the small inhibitors PHA-665752 or JNJ38877605.
Results: Resistance was sustained by: (i) an initial *MET* gene amplification, leading to increased protein expression and constitutive phosphorylation, (ii) a subsequent amplification and overexpression of wild-type *KRAS* with activation of the MAPK pathway. Cells harboring *KRAS* amplification became MET-independent and underwent an "oncogene addiction switch". The resistance was unstable, since cells progressively lost *MET* and *KRAS* extra-copies after drug withdrawal, reacquiring sensitivity to MET inhibitors.
Conclusions: This is one of the first pre-clinical study highlighting mechanisms of resistance to long-term exposure to selective MET kinase inhibitors and showing that amplification of *MET* and *KRAS* genes mediate resistance to MET kinase inhibitors. These findings provide insights to strategies for preventing and/or overcoming drug resistance.

313 POSTER

Resistance to the microtubule-stabilizing agents, peloruside A and laulimalide, is associated with multiple β-tubulin alterations

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Peloruside A (peloruside) and laulimalide are microtubule-stabilizing agents that are effective at nanomolar concentrations in cultured cancer cells. The drugs have a mechanism of action similar to that of paclitaxel, a clinically useful anticancer drug, but have a number of advantages that make them unique to paclitaxel. They also bind to a distinct site on tubulin that differs from the classical taxoid site. The development of chemoresistance in cancer cells is a major problem to the successful treatment of cancer in the clinic. The role of β-tubulin alterations in the resistance to taxoid-site antimicrotubule agents has been reported previously. In an attempt to understand the mechanisms of resistance to peloruside and laulimalide, we used two drug-resistant sublines (R1 and L4) of the 1A9 human ovarian carcinoma cell line. The R1 and L4 cells differ from each other in their resistance ratio to peloruside and laulimalide, with R1 being less resistant (6.9- and 1.8-fold, respectively) and L4 being more resistant (20.1- and 29.5-fold, respectively). The cells exhibit a βI-tubulin mutation at amino acid positions 296 (in R1) and 306 (in L4). To determine the role of tubulin alterations in the resistance profile of the cells, we examined: (1) the ability of peloruside/laulimalide to stabilize microtubules in the resistant cells using confocal microscopy; (2) the alterations in β-tubulin isotype expression using quantitative real-time PCR, Western blotting, immunocytochemistry, and 2-dimensional gel electrophoresis; and (3) the functional significance of the altered β-tubulin isotype expression using small-interfering RNA technology (siRNA). Confocal microscopy showed that there was an impaired ability of peloruside and laulimalide to bind and stabilize microtubules in the resistant cells. In L4 cells, an increased mRNA and protein expression of βII- and βIII-tubulin isotypes was observed. Interestingly, siRNA-mediated knock-down of both βII- and βIII-tubulin partially sensitized the L4 cells to laulimalide, indicating that changes in isotype expression may be important in the development of resistance by the cells. Thus, our data show that overexpression of βII- and βIII-tubulin isotypes, in addition to a βI-tubulin mutation, play a vital role in cancer cell's resistance to peloruside and laulimalide. This information will be helpful for improving the design and targeting of microtubule-active anticancer drugs.

314 POSTER

Acceleration of migration mediated by Insulin-like Growth Factor-1 receptor and Syk kinase in bortezomib-resistant myeloma cells

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Purpose: The type 1 IGF receptor (IGF1R) is a transmembrane tyrosine kinase, which is overexpressed by several tumors, and mediates proliferation and apoptosis protection for tumor cells. IGF signaling affects hypoxia signaling, protease secretion, tumor cell motility, and adhesion. ProIGF1R is cleaved into alpha and beta chains by processing, and their heterodyne moves onto plasma membrane. Folding after transfer to Golgi apparatus modifies the beta chain, and degradation by ubiquitin-proteasome system occurs. Bortezomib is a proteasome inhibitor for multiple myeloma, and expression of chaperon proteins such as IGF1R could be increased by bortezomib. Here, we found crosstalk between IGF1R and Syk kinase in bortezomib-resistant myeloma cells, and IGF1R might be related to cell migration. Inhibition of IGF1R activity might lead to blockade of cell invasion in bortezomib-resistant myeloma.
Methods: IGF1R expression of myeloma cell line IM-9 and bortezomib-resistant IM-9 was examined with or without treatment of bortezomib by flow cytometric and western blot analyses. The IGF1R and Syk kinase genes were introduced into 293T cells, and binding assay was performed by co-immunoprecipitation. Migration assay in parent IM-9 and bortezomib-resistant IM-9 cells was preformed in the presence or absence of IGF1 and IGF2.
Results: The IGF1R expression on bortezomib-resistant cells was stronger than parent IM-9 cells by flow cytometric and Western blot analyses. When IGF1R and Syk kinase genes were introduced into 293T cells, and IGF1R and Syk kinase were co-immunoprecipitated. The bortezomib-resistant IM-9 cells migrated more than parent IM-9 cells in the presence of IGF1 and IGF2.
Conclusion: Stronger expression of IFG1R on bortezomib-resistant IM-9 cells was observed, and this might be related to cell migration and invasion mediated by Syk kinase. Inhibition of IGF1R activity might lead to blockade of cell invasion in bortezomib-resistant myeloma.