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.

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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.