of the NF-κB subunit p65 reversed the increased chemosensitivity of HIF-1α-deficient cells.
Conclusions: In summary, we identified HIF-1α as a potent regulator of p53 and NF-κBactivity under conditions of genotoxic stress. We conclude that *p53* mutations in human tumors hold the potential to confound the efficacy of HIF-1-inhibitors in cancer therapy.

304 POSTER

Anti-angiogenic therapy improves response rate in erlotinib resistant NSCLC xenografts

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Non small cell lung cancer (NSCLC) is one of the leading causes of cancer deaths in the Western world. Combination chemotherapy is the therapeutic option for advanced diseases but with limited efficacy. Targeted therapies entered the clinics and led to a minor improvement of survival. However, many patients do not benefit from cytotoxic agents or targeted therapies. Therefore, reliable markers to select treatments for patients most likely to respond are in urgent need.
In our study, 25 patient-derived NSCLC xenografts were established and characterized. They revealed a high degree of similarity with the original tumor concerning histology, immunohistochemistry as well as gene profiling. The responsiveness to four cytostatics drugs (etoposide, carboplatin, gemcitabine, paclitaxel) and two epidermal growth factor receptor inhibitors (erlotinib and cetuximab) was evaluated in these xenografts according to clinical criteria.
The RNA expression profile of the xenografts was analyzed with the GeneChip HGU133Plus2.0. The data were evaluated statistically with the help of GeneSpring GX 11.0.
Within a class comparison, more than 2500 probe sets were found to be differentially expressed between erlotinib responders (2 xenografts) and non-responders (23 xenografts).
Differentially expressed genes were vascular endothelial growth factor a (VEGFA) and neuropilin and tolloid-like 2 (NETO2). VEGFA was higher expressed in the erlotinib resistant tumors. It may cause a better vascularization and thus result in a better survival of the tumor. The results were validated with TaqMan-PCR. With a combination therapy of erlotinib and bevacizumab the response rates could substantially be improved in the erlotinib resistant tumors.
In conclusion, a differential gene expression pattern was found in our patient derived xenografts allowing the identification of rational combination therapies. The patient-derived xenograft system offers a valuable tool to investigate targeted therapies and biomarker regulations in a clinically related way.

305 POSTER

Targeting glioblastoma stem cells: overcoming temozolomide resistance by ALDH1 inhibition

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Glioblastoma (GBM), the most frequent brain tumor of adults is still associated with a poor prognosis. Despite various efforts to improve postoperative therapeutic regimens in recent times, relapse occurs regularly. The chemoresistance of malignant gliomas might be caused by a barely characterized tumor stem cell subpopulation residing in a specific tumor microenvironment. Besides others, hypoxia may play a critical role in inducing a resistant tumor cell type. While normal brain tissue shows an oxygen partial pressure (pO_2) of 24−27 mmHg, gliomas feature an average pO_2 of only 13 mmHg.
So far, temozolomide (TMZ) is the gold standard of care for newly diagnosed glioblastoma. Recently we could show that aldehyde dehydrogenase 1 (ALDH1) positive glioblastoma cells show brain tumor stem cell capacity. In the current investigation we examined the impact of ALDH1 expression on GBM temozolomide resistance. In vitro cytotoxicity was evaluated by colorimetric MTT- and colony formation assays. Flow cytometry was used to analyze the amount of apoptotic cells. Neurosphere formation in neurobasal medium and differentiation experiments were applied to identify tumor stem cells. Furthermore, ALDH1 expression and temozolomide resistance was correlated with the MGMT status of various established and primary cell lines.
Sensitivity to temozolomide in resistant established and primary glioma cell lines was achieved by inhibition of ALDH1 with 4-diethylaminobenzaldehyde (DEAB). A specific knock down of ALDH1 by siRNA confirmed these findings. Under hypoxic conditions the cytotoxic effect of temozolomide was strongly attenuated but could be restored by ALDH1 inhibition. In the present study we show that ALDH1 is strikingly upregulated under hypoxic conditions, potentially leading to an increase of chemoresistance in gliomas. Hypoxia inducible factors (HIF1 alpha, HIF2 alpha) are involved in the regulation of ALDH1. Since post-therapeutic relapse is most probably due to a stem cell subfraction within the tumor bulk, special interest should be drawn to these cells. After combination therapy of temozolomide and DEAB, glioma cells were no longer able to proliferate in stem cell promoting medium or to form neurospheres; the remaining cells lost their undifferentiated stem cell-like phenotype.
In conclusion we suggest ALDH1 as an important prognostic factor in glioma care. Combination therapy of temozolomide with ALDH1 inhibitors might strongly improve clinical outcome of GBM patients.

306 POSTER

The selective reduction in the production of Bmi-1 protein leads to tumor growth control in multiple tumor models

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Background: Elevated expression of Bmi-1, a polycomb protein (also called PCGF4), is correlated with the chemo- and radio-resistance of a sub-fraction of tumor cells that also demonstrate stem cell characteristics. These stem-like cells are thought to be responsible for tumor recurrence leading to treatment failures in many cancer types. Bmi-1 has been shown to play a significant role in many neoplasias, particularly in glioblastoma where there is compelling evidence that Bmi-1 over-expression in glioblastoma multiforme (GBM) is a key event for tumor growth and intrinsic chemo-resistance.
Results: PTC has identified low molecular weight compounds that potently and selectively inhibit the production of Bmi-1 protein. A subset of these compounds act by targeting the post-transcriptional regulation of Bmi-1 synthesis, which reduces the translation rate of Bmi-1. This occurs both in cancer cells in culture and in various xenograft tumor models. The loss of Bmi-1 expression induced by these molecules leads to the reduction in global levels of mono-ubiquitinated histone 2A and causes either apoptosis or senescence in tumor cells. In murine xenograft tumor models, these compounds reduce intratumor Bmi-1 protein levels by up to 70% and tumor growth by up to 50% when administered orally as single agent therapy. The in vivo evaluation of activity is in progress where lead molecules are used in combination with standard-of-care cytotoxics.
Conclusions: Results from our studies support the hypothesis that targeting the production of the stem cell protein Bmi-1, known to be important for tumor cell survival and resistance, may enhance treatment success and improve patient outcomes.
This program is funded by SDDI award #092687 from the Wellcome Trust.

307 POSTER

An antisense molecule to HER3 sustains growth inhibitory effects in gefitinib resistant cells that are independent of MET overexpression

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Although HER3 is not typically amplified or overexpressed in many tumor cells lines like EGFR or HER2 family members, HER3 is emerging as a critical family member since (1) it is a key link to the PI3K pathway for HER family members, (2) it can heterodimerize with HER1 and HER2, and (3) it can be activated via autocrine signaling by binding its cognate ligand, heregulin. These features help explain why increased activation of HER3 can mediate resistance to HER1 and HER2 inhibitors such as gefitinib, lapatanib, or Herceptin.
We have been attempting to understand the basis of unusual sensitivity of the lung carcinoma cell line, HCC827 to gefitinib (IC_{50} ~10 nM) and acquired resistance mechanisms after the cells were chronically exposed to increasing concentrations of gefitinib in vitro. The resistant cell lines were independently selected and are distinct from that reported to be driven by HER3 hyperactivation associated with MET amplification (Engelman et al., Science. 2007 316: 1039). The cell lines were highly resistant to gefinitib (IC_{50} >10 μM) but unlike past reports, the intracellular pEGFR levels were dramatically reduced while pHER3 and HER3 levels were unchanged when compared to HCC827 parental cells. Furthermore, no alteration in MET expression has been detected. Despite the lack of a HER3 activation signature, the resistant clones were equally or more sensitive to the treatment of an antisense molecule against HER3, designated EZN-3920. The parental HCC827, as well as the resistant cell lines were the most sensitive cells to EZN-3920 compared with 20 other cell lines. Furthermore,

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.
This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare and family Affairs, Republic of Korea (A010250).

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.
Supported by grants NHRI-EX99–9911BC, NTU99R311002, and NSC99–3112-B-002–038.

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