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

U. Jo[1], J.S. Sung[2], H.Y. Jung[3], K.H. Park[4], H.N. Jung[3], Y.M. Whang[3], Y.H. Kim[3]. *[1]Korea Univ. Anam Hospital,Genomic Research Center for Lung and Breast/Ovarian Cancers, Department of Internal Medicine and Division of Brain Korea 21 Project for Biomedical Science, Seoul, Korea; [2]Korea Univ. Anam Hospital, Department of Internal Medicine and Division of Brain Korea 21 Project for Biomedical Science, Seoul, Korea; [3]Korea Univ. Anam Hospital, Genomic Research Center 3712, Seoul, Korea; [4]Korea Univ. Anam Hospital, Division of Oncology/Hematology Department of Internal Medicine, Seoul, Korea*

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

D. Ou[1], C. Hsu[1], Y.T. Huang[1], Y.C. Shen[1], B.S. Lee[1], A.L. Cheng[1]. *[1]National Taiwan University Hospital, Oncology, Taipei, Taiwan*

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

R.C.C. Chai[1], J.L. Vieusseux[1], C.H. Nguyen[1], B.J. Lang[1], M.M. Kouspou[1], J.T. Price[1]. *[1]Monash University, Biochemistry and Molecular Biology, Melbourne Victoria, Australia*

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

E.S.R. Collie-Duguid[1], J.D. Bissett[2], R.D. Petty[1], M.C. Nicolson[2], K. Kerr[3], L. Cao[1]. *[1]University of Aberdeen, School of Medicine and Dentistry, Aberdeen, United Kingdom; [2]NHS Grampian, Department of Oncology, Aberdeen, United Kingdom; [3]NHS Grampian, Department of Pathology, Aberdeen, United Kingdom*

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