Thursday, 22 March 2012 12:30–13:30

POSTER SESSION

Pharmacology, New Drug Development

294 Poster discussion

Combining Everolimus with a PI3K Inhibitor Mitigate Cross-Talk and Improves Response to Endocrine Therapy in a Pre-Clinical Breast Cancer Model

K. Shen[1], M. Zhao[1], X. Chen[1], X. Sun[2], J. Wang[2]. [1]*Ruijin Hospital Shanghai Jiaotong University School of Medicine, Comprehensive Breast Health Center, Shanghai, China;* [2]*School of Medicine Shanghai Jiao-tong University, Institute of Medical Sciences, Shanghai, China*

Background: The mechanism of endocrine therapy (ET) resistance in breast cancer remains unclear. Everolimus (EVE), an oral mTOR inhibitor, can reverse ET resistance concurrent treatment with tamoxifen (TAM), letrozole or exemestane. However, increasing evidence suggests that EVE may cause a cross-talk pathway activation, which induced Akt phosphorylation, and therefore decreased the treatment efficacy. In this study, we assessed whether EVE combination with a PI3K inhibitor (LY294002) can inhibit this pathway cross-talk activation in breast cancer.

Material and Methods: Breast cancer lines (MCF-7 and BT474) were treated with TAM alone, TAM+EVE, TAM+LY294002, TAM+EVE+LY294002, or control in vitro. Cell viability, division cycle and apoptosis were analyzed by flow cytomentry. PI3K/Akt/mTOR signaling pathway and a potential downstream target (HIF-1a) status was evaluated by western blot assay. VEGF level was detected by ELISA.

Results: EVE had synergistic effects with TAM. TAM+EVE+LY294002 treatment had best antitumor effect compared with TAM alone or other two agents combination group (TAM+EVE; TAM+ LY294002), and more tumor cell apoptosis was observed in this three agents group compared with others. In addition, we found cross-talk phosphorylation of Akt (pAkt) expression increased in EVE alone or TAM+EVE group, even though pathway downstream biomarkers (P70S6k and 4EBP1) expression decreased. However, adding LY294002 to the TAM+EVE treatment can significantly decreased pAKT expression as well as the phosphorylation level of P70S6K and 4EBP1. Moreover, in hypoxic condition, HIF-1a expression increased in TAM alone treatment group, which can significantly inhibited by adding EVE treatment, especially in combination with LY294002. In addition, MMP-3 level can also be decreased in this three agents group, and VEGF level followed the same trend.

Conclusion: Combining EVE with a PI3K inhibitor (LY294002) can reverse the pathway related cross-talk pAkt activation, resulting with enhancement of ET efficacy. These promising results warrant further study in breast cancer treatment.

295 Poster

Feasibility Examination of Prior Administration of Cyclophosphamide in TC Combination

H. Abe[1], Y. Kawai[1], T. Mori[1], H. Cho[1], Y. Kubota[1], T. Umeda[1], Y. Kurumi[2], T. Tani[3]. [1]*Shiga University of Medical Science Hospital, Division of Breast and General Surgery, Otsu Shiga, Japan;* [2]*Shiga University of Medical Science, Department of Comprehensive Surgery, Otsu Shiga, Japan;* [3]*Shiga University of Medical Science, Department of Surgery, Otsu Shiga, Japan*

Background: TC (docetaxel 75 mg/m^2 and cyclophosphamide 600 mg/m^2 q3w) combination is used for neoadjuvant/adjuvant chemotherapy in primary breast cancer. The incidence of allergic reaction is reportedly more common in patients who receive docetaxel before cyclophosphamide. This study aimed to determine the significance cyclophosphamide and docetaxel sequence.

Methods: A prospective analysis was performed in 49 consecutive patients treated with TC for stage I-IIB breast cancer from March 2010 to June 2011. Premedication was administered with granisetron, dexamethasone and chlorpheniramine. Patient charts were reviewed for completion rate and adverse events. Two-tailed Fisher exact test was used to evaluate the adverse events between cyclophosphamide and docetaxel sequence.

Results: Of 49 patients, 26 received docetaxel prior to cyclophosphamide, and 23 received cyclophosphamide before docetaxel. There were no differences in patient characteristics between the two groups. Completion rates were 95.6% in the prior cyclophosphamide group, and 100% in the prior docetaxel group. The relative dose intensities of docetaxel and cyclophosphamide were 94.5% and 94.8% in the prior cyclophosphamide group, and 98.5% and 98.7% in the prior docetaxel group ($p < 0.01$). In the prior cyclophosphamide group, severe neutropenia occurred in 96% of patients, but in only 46% of patients in the prior docetaxel group ($p < 0.01$). Significantly fewer cases of eczema (27% vs. 61%), nausea (8% vs. 48%), stomatitis (23% vs. 61%), and diarrhea (4% vs. 30%) were observed in the prior docetaxel group as compared with the prior cyclophosphamide group ($p < 0.01$). Decreased incidences of fatigue (50% vs. 65%) and edema (19% vs. 35%) were found in the prior docetaxel group ($p < 0.05$). No difference was observed in allergic reaction or neuropathy between the two groups.

Conclusion: Patients receiving cyclophosphamide prior to docetaxel were at increased risk of several toxicities as compared with patients receiving docetaxel prior to cyclophosphamide in TC combination therapy.

296 Poster

Regulator of G Protein Signaling 6 (RGS6) Suppresses Mammary Tumorigenesis by Enhancing DNA Damage Signaling and Blocking Oncogenic Transformation

B. Maity[1], J. Huang[1], A. Stewart[1], Y. O'Malley[2], J. Hagen[1], R. Askeland[3], D. Quelle[1], S. Sugg[2], R. Fisher[1]. [1]*University of Iowa College of Medicine, Pharmacology, Iowa City, USA;* [2]*University of Iowa College of Medicine, Internal Medicine, Iowa City, USA;* [3]*University of Iowa College of Medicine, Pathology, Iowa City, USA*

RGS6 is a member of the RGS protein family that function as negative regulators of G protein signaling by virtue of their GTPase-activating protein (GAP) activity toward Ga subunits. Given the link between persistent G protein activity and cancer and a reduced risk of bladder tumor formation in humans expressing an RGS6 SNP that increases its translation, we hypothesized that RGS6 might function as a tumor suppressor. Here we show that RGS6 was expressed exclusively in ductal epithelial cells in mammary tissue and its expression is markedly down-regulated in human breast cancer with loss of RGS6 correlating with malignancy. Expression of RGS6 in human breast cancer cells provoked impressive anti-proliferative actions and induction of apoptosis by p53-independent mechanisms. RGS6 activated the intrinsic pathway, involving regulation of Bax/Bcl-2, cytochrome C release and activation of caspase-3 and -9. RGS6 promoted loss of mitochondrial membrane potential ($\Delta\Psi$m) and increases in reactive oxygen species (ROS). RGS6-induced ROS mediated its ability to promote caspase activation and loss of $\Delta\Psi$m, suggesting a feed forward amplification mechanism of ROS in RGS6-induced apoptosis. The pro-apoptotic actions of RGS6 are independent of its GAP activity, thus defining an entirely novel signaling activity of RGS6. We next employed RGS6$^{-/-}$ mice to interrogate the role of RGS6 in breast tumor progression. Mice lacking RGS6 exhibited accelerated tumor formation and increased tumor size compared to WT mice in response to the carcinogen DMBA, providing new evidence that RGS6 functions as a suppressor of breast tumorigenesis. Also, nearly 20% of virgin female RGS6$^{-/-}$ mice over 1 year of age developed spontaneous breast tumors in the absence of DMBA treatment. We recently reported that RGS6 is required for doxorubicin-induced ATM and p53 activation. RGS6$^{-/-}$ mice also exhibit increased DMBA-induced DNA damage due to loss of ATM/p53 mediated DNA repair, underscoring the importance of RGS6 in DMBA-induced DNA damage signaling. Further, we found that RGS6 dramatically suppresses oncogene-induced cellular transformation of MEFs mediated by combined expression of constitutively active Ras and dominant negative p53. These results demonstrate that RGS6 is a critical regulator of both the cellular responses to DNA damage and oncogene activation and that RGS6 functions as a tumor suppressor *in vivo*. Thus, RGS6 represents a novel target in the treatment of breast cancer.

297 Poster

Efficacy and Safety of Fetal Human Estrogen Estetrol (E4) in Women with Estrogen-receptor Positive Early Breast Cancer

C. Singer[1], C. Natter[1], S. Steurer[2], M. Rudas[3], F. Moinfar[4], N.R.C.A. Appels[5], M. Visser[5], E. Kubista[6], H.J.T. Coelingh Bennink[7]. [1]*Medical University of Vienna, Dept of Senology, Vienna, Austria;* [2]*Otto Wagner Spital, Dept of Pathology, Vienna, Austria;* [4]*MUGraz, Dept of Pathology, Graz, Austria;* [5]*Pantarhei Biosciences, Zeist, The Netherlands;* [7]*Pantarheibioscience, Zeist, The Netherlands*

Background: Estetrol (E4) is a natural fetal estrogen which exerts estrogenic effects on reproductive organs and on the bone, and effectively reduces menopausal smyptoms. In contrast to other estrogens, however, E4 has estrogen-antagonistic effects on breast cancer cell lines *in vitro* and in the rat DMBA model, which would make it a suitable Hormone Replacement Therapy (HRT) in breast cancer patients, particularly in women who are being treated with aromatase inhibitors.

Patients and Methods: We have investigated the effect of 14 days pre-operative treatment with 20 mg E4 per day on tumor proliferation, apoptosis,