the biological activity of the Ras proteins, the key molecular pathways downstream of K-Ras involved in promoting lung tumorigenesis need to be identified in order to provide better targets for lung cancer therapy. We hypothesize that a crucial target of oncogenic K-Ras in the lung is nuclear factor kappa B (NFκB). NFκB is a dimeric transcription factor that promotes proliferation, mediates resistance to apoptosis, and is abnormally activated in a large variety of human malignancies.

**Methods:** In order to analyze the role of NFκB downstream of K-Ras in the airway epithelium, a tetracycline-inducible system was used to stably and inducibly express either the wild type form of K-Ras (K-RasWT) or the oncogenic form (K-RasV12) in the lung adenocarcinoma H1703 cell line. We also analyzed primary human bronchial cells that were either immortalized (AALEB) or immortalized and transformed with K-RasV12 (AALEB-K-Ras). NFκB activity in these cells was analyzed by western blotting, electrophoretic mobility shift assays, luciferase assays and expression of NFκB target genes by real-time PCR.

**Results:** Expression of K-Ras-WT or K-RasV12 in H1703 cells after tetracycline induction was accompanied by both increased expression of NFκB target genes and increased NFκB DNA binding activity. DNA binding was mediated primarily by p50 and p65 NFκB subunits. Although NFκB luciferase reporter activity was detectable prior to K-Ras expression, expression of K-Ras-V12, but not K-Ras-WT, enhanced NFκB activity. Interestingly, K-RasV12 does not activate NFκB by the canonical NFκB activation pathway; in both H1703 and AALEB cells expressing K-RasV12, there was no increase in IkappaBalpha (IκBα) phosphorylation/degradation. Nonetheless, activation seems to be dependent on Ikappab Balpha (Iβcβ) phosphorylation/degradation. Nonetheless, activation seems to be dependent on IkappaB kinase (IKK) activity for the following reasons: (1) When compared to AALEB cells, AALEB K-Ras cells displayed increased IKK phosphorylation levels; and (2) specific inhibition of IKKβ activity in H1703 cells by the novel pyridine derivative compound 65-1942 (obtained under MTA agreement with Bayer) supressed NFκB activity. Finally, as no increase in p65 phosphorylation at S536 was observed in either cell type expressing K-RasV12, the mechanism of NFκB activation does not involve direct phosphorylation of p65 at S536 by IKK.

**Conclusions:** We have determined a causal link between K-RasV12 expression and NFκB activation in lung epithelial cells in vitro. NFκB in these cells is not activated either by the canonical pathway of IκBα phosphorylation/degradation or by p65 phosphorylation at S536. Although the specific mechanism of activation is being investigated both in H1703 and in primary AALEB cells, we have shown that it depends on IKK. IKKβ inhibitors, therefore, represent a potentially promising new therapeutic direction in lung cancer. Studies are underway to confirm their efficacy in a pre-clinical lung cancer mouse model.

**P-116**

**Increase of carcinogenic risk via enhancement of cyclooxygenase-2 expression and**

Chang, Louis W.1 Chang, Yun-Ching2 Ho, Chia-Chi3 Tsai, Ming-Hsien4 Lin, Pinpin2
1 DEHOM, National Health Research Institutes, Zhunan, Taiwan 2 Graduate institute of Basic Medical Science, China Mediacal University, Taichung, Taiwan 3 DEHOM, National Health Research Institutes, Miaoli County, Taiwan

Animal studies demonstrated that females are more susceptible than males to benzo[α]pyrene (BaP)-induced toxicities, including lung carcinogenesis. Elevation of cyclooxygenase-2 (COX-2) expression has been shown to increase the risk of cancer development. BaP induces COX-2 expression, and an interaction between BaP and estrogen in relation to COX-2 expression is expected. In the present study, 10 mM BaP alone only slightly increased COX-2 mRNA expression and 10 nM 17-beta estradiol (E2) alone slightly increased prostaglandin E2 (PGE2) secretion in human bronchial epithelial cells. However, co-treatment with BaP and E2 potentiated COX-2 mRNA expression and significantly elevated PGE2 secretion. Utilizing specific inhibitors and reporter assays, we further investigated the potentiation mechanisms of E2 on BaP-induced COX-2 expression. First, E2 activated estrogen receptor to increase PGE2 secretion, which directly increased binding sites. The aim of this study is to investigate the combination effects of gefitinib plus four commonly used chemotherapeutic agents for the treatment of non-small cell lung cancer (NSCLC) in a gefitinib-insensitive lung cancer cell line and its adriamycin-induced multi-drug resistant subclones.

**Methods:** NCI-H23 was a lung adenocarcinoma cell line with wide type EGFR gene expression. Its high P-glycoprotein (Pgp) expression subclones H23 adr 0.1 and H23 adr 0.3 were developed by stepwise exposure to escalating concentrations of adriamycin. The expression levels of (Pgp), breast cancer resistant protein (BCRP), and multidrug resistant protein-1 (MRP-1) were checked by RT-QPCR and immunoblot. The combination effects of gefitinib plus single chemotherapeutic agents including paclitaxel (Pac), docetaxel (Doc), vinorelbine (NVB) and gemcitabine (Gem) were evaluated by using the tetrazolium dye colorometric assay with application of the classical isobole methods in these cell lines. To evaluate the inhibition of ABC transporters-mediated drugs efflux, Rhodamine 6G was used as the fluorescence probe for evaluating the inhibitory effects of gefitinib (0-10 μM) on the ABC transporters.

**Results:** We found that H23 Pgp subclones showed increased resistance to three antitubulins, Pac, Doc and NVB, but not to Gem and gefitinib. Gefitinib could partially reverse Pgp related Pac, Doc and NVB resistance in a dose-dependent fashion, showing marked synergistic effects, while gefitinib showed an additive combination effect with Gem. There was no statistical difference in the values of combination index between gefitinib plus each antitubulins. Rhodamine 6G-efflux assay in the tested cell lines revealed that fluorescence-efflux was inhibited with fluorescence accumulation by gefitinib in a dose-dependent fashion.

**Conclusions:** Our results showed that gefitinib could enhance cytotoxicities of anti-tubulines in Pgp expressing NSCLC cells which showed wild type EGFR. Our results provide a rationale to test these combinations as salvage treatment in NSCLC patients who failed prior antitubulin treatment and also in patients whose tumors express high Pgp.

**P-117**

**BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

**Gefitinib enhanced antitubulin cytotoxic effects in high P-glycoprotein and wild epidermal growth factor receptor expressing non-small cell lung cancer cells by inhibiting ATP binding cassette transporters**

Chang, Kuo-Ting Hsiao, Shih-Yin Chiu, Fan-Hsiang Tsai, Chun-Ming Section of Thoracic Oncology, Chest Department, Taipei Veterans Gen. Hospital, Taipei, Taiwan

**Background:** Gefitinib, a selective tyrosine kinase inhibitor (TKI) of epidermal growth factor receptor (EGFR), may inhibit functions of ATP-binding cassette (ABC) transporters by competing against the ATP binding sites. The aim of this study is to investigate the combination effects of gefitinib plus four commonly used chemotherapeutic agents for the treatment of non-small cell lung cancer (NSCLC) in a gefitinib-insensitive lung cancer cell line and its adriamycin-induced multi-drug resistant subclones.

**Methods:** NCI-H23 was a lung adenocarcinoma cell line with wide type EGFR gene expression. Its high P-glycoprotein (Pgp) expression subclones H23 adr 0.1 and H23 adr 0.3 were developed by stepwise exposure to escalating concentrations of adriamycin. The expression levels of (Pgp), breast cancer resistant protein (BCRP), and multidrug resistant protein-1 (MRP-1) were checked by RT-QPCR and immunoblot. The combination effects of gefitinib plus single chemotherapeutic agents including paclitaxel (Pac), docetaxel (Doc), vinorelbine (NVB) and gemcitabine (Gem) were evaluated by using the tetrazolium dye colorometric assay with application of the classical isobole methods in these cell lines. To evaluate the inhibition of ABC transporters-mediated drugs efflux, Rhodamine 6G was used as the fluorescence probe for evaluating the inhibitory effects of gefitinib (0-10 μM) on the ABC transporters.

**Results:** We found that H23 Pgp subclones showed increased resistance to three antitubulins, Pac, Doc and NVB, but not to Gem and gefitinib. Gefitinib could partially reverse Pgp related Pac, Doc and NVB resistance in a dose-dependent fashion, showing marked synergistic effects, while gefitinib showed an additive combination effect with Gem. There was no statistical difference in the values of combination index between gefitinib plus each antitubulins. Rhodamine 6G-efflux assay in the tested cell lines revealed that fluorescence-efflux was inhibited with fluorescence accumulation by gefitinib in a dose-dependent fashion.

**Conclusions:** Our results showed that gefitinib could enhance cytotoxicities of anti-tubulines in Pgp expressing NSCLC cells which showed wild type EGFR. Our results provide a rationale to test these combinations as salvage treatment in NSCLC patients who failed prior antitubulin treatment and also in patients whose tumors express high Pgp.
COX-2 expression. Second, E2 potentiated BaP-induced nuclear factor-kB (NF-kB) activation, which regulates COX-2 expression. Third, although the aryl hydrocarbon receptor (AhR) did not play a role in BaP-induced COX-2 expression, the potentiation effect of E2 itself was AhR dependent. We further demonstrated that BaP induced the production of genotoxic E2 metabolites (2- and 4-hydroxyestradiols) via AhR-up-regulated cytochromes P450 1A1 and 1B1. These metabolites could directly activate NF-kB to further promote COX-2 mRNA expression in human lung epithelial cells. These findings were further supported by increased PGE2 secretion in rat lung slice cultures. Our findings that the BaP-VE2 interaction enhanced COX-2 expression and hydroxyestriadiol accumulation in the media of cultivated lung cells and tissues provide the needed scientific basis for higher risk of BaP-associated lung cancer in females.

P2-118
Five-bromodeoxyuridine induces differentiation of a human small cell lung cancer cell line is associated with alteration of gene expression
Chen, Yuan; Pacyna-Gengelbach, Manuela; Deutschmann, Nicole; Ye, Fei; Petersen, Iver
Institute of Pathology, University Hospital Charite, Berlin, Germany
Small cell lung carcinoma (SCLC) is a highly metastatic disease with a poor prognosis due to its resistance to current modes of therapy. SCLC appears to arise from neuroendocrine cells with the potential to differentiate into a variety of lung epithelial cell lineages. In order to investigate molecular events underlying the cell type transition in SCLC, we established a differentiation cell model by modification of a SCLC cell line H526 with a differentiation inducing agent 5-bromodeoxyuridine (BrdU). The BrdU treatment led to a dramatic conversion from a suspension cell line H526 to an adherent cell line variant H526B exhibiting an epithelioid phenotype. DNA fingerprinting by random amplified polymorphic DNA (RAPD) method showed an identical DNA binding pattern between H526 and H526B, indicating that the H526B subpopulation arose from the original cell line H526 and the contamination from other cell types can be ruled out. The BrdU modified cells H526B remarkably reduced the ability of colony formation in soft agar and suppressed the tumor growth rate in immune-deficient nude mice. The phenotypic transition was consistent with upregulation of several lung cancer differentiation markers such as surfactant protein C (SFTPC), thyroid transcription factor 1 (TTF-1), Connexin 26 (Cx26), insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1), as well as homeobox genes like LAGY, PITX1 and HOXB2. Our data suggest that BrdU induced cell differentiation could be linked to the development of a less aggressively phenotype in small cell lung cancer.

All-trans retinoic acid can restore transforming growth factor-β tolerance in non-small cell lung cancer cell lines through inhibition of Smad1/5/8
Choi, Hye-Soon1 Young Mi, Whang1 Kim, Tae Woo2 Kim, Seok Jin3 Kim, Yeul Hong4
1 Genomic Research Center for Lung and Breast/Ovarian Cancers, Seoul, Korea 2 Graduate School of Medicine, Korea University, Seoul, Korea 3 Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea 4 Genomic Research Center for Lung and Breast/Ovarian cancers, Department of Internal Medicine, Korea University College of Medicine, Brain Korea 21 Program for Biomedical Sciences, Seoul, Korea
Transforming growth factor-β (TGF-β) is an essential regulator of cellular physiological processes including proliferation, differentiation and angiogenesis.

Retinoids have been shown to possess differentiation, antiproliferative and apoptosis-inducing properties in cancer cells. We have studied the effects of all-trans retinoic acid (ATRA) after treated with different levels of TGF-β1 in non-small cell lung cancer cell lines (NSCLCs). In addition, we examined whether ATRA effects in signal pathway of NSCLCs. We analyzed expression of TGF-β receptor I and II (TGF-βRI, TGF-βRII) by RT-PCR and treated with ATRA and a variety of concentrations of TGF-β1 for different durations in NSCLCs. In addition, the time-dependent cytotoxic effects of ATRA alone, TGF-β1 alone and combination were studied by the MTT and cell counting assays. We carried out western blot analysis to evaluate whether combined treatment affects the expression of Smads or TGF-β RI (ALK5, ALK1). TβRII/ALK5 complex activates Smad2/3 pathway and TβRII/ALK1 complex activates Smad 1/5 pathway. These pathways have been known to be associated with cellular growth inhibition through induction of phosphorylated Smad2; whereas cellular proliferation through induction of phosphorylated Smad1/5 in cancer cells.

The results showed that TGF-β1 alone increased cellular growth of NSCLCs, and the combined treatment of ATRA and TGF-β1 inhibited the growth of NSCLCs. The combined treatment of ATRA and TGF-β1 reduced TGF-β1-induced phosphorylated Smad1/5/8. However, the total protein levels of ALK5 and ALK1 were not changed by the combined treatment of ATRA and TGF-β1. In addition, the combined treatment of ATRA and TGF-β1 reduced migration compared to the treatment with TGF-β1 in A549 and H1299 cell lines.
Therefore, these findings suggest that treatment with ATRA reduced the growth and migration of NSCLCs by inhibition of TGF-β1-induced phosphorylated Smad1/5/8 which caused growth progression and migration. ATRA may have a potential therapeutic role in the treatment of lung cancer patients with increased levels of TGF-β1.

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