found in the amplified cell lines. This amplification confers sensitivity of NSCLC to inhibitors of the tyrosine kinase activity of c-Met. To further determine the molecular characteristics of cell lines carrying c-Met amplification, we investigated the levels of downstream effectors of c-Met, such as phospho paxillin, phospho-extracellular signal-regulated kinase and phospho-S6 proteins. Interestingly, S6 kinase a serine-threonine kinase whose activation is thought to regulate a wide array of cellular processes involved in the mitogenic response including protein synthesis, translation of specific mRNA species, and cell cycle progression from G1 to S phase was more frequently activated in cell lines with gene amplification of c-Met than in their wild-type counterparts. c- Met amplification may thus identify a subset of lung cancers that are uniquely altered signaling which potentially sensitive to c-Met inhibition.

P2-126 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

The role of pax transcription factors in lung carcinogenesis: relationship to c-Met receptor tyrosine kinase

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Background: Lung cancer still remains one of the leading causes of all cancer related deaths. They can be divided into two major types, non small cell (NSCLC) and small cell (SCLC) lung cancers. Amongst the two, prognosis in patients with NSCLC is better compared to those with SCLC, however the overall survival rate is still very low (17%). A plethora of molecular changes due to various genomic alterations are known to contribute to the development and spread of lung cancers, for instance mutations in p53, RB, Ras, several receptor and non-receptor tyrosine kinases are known to contribute to the variety of phenotypes seen in lung cancer. One of the long term goals of our lab is to map and study the biological significance of loss and gain of function mutations in various signaling molecules in lung cancer; with the idea of developing novel therapeutics. In this regard, we examined the role of Pax transcription factors in lung cancer. To date, the Pax family consists of nine members that are all characterized by the presence of a paired domain. They are indispensable for various developmental processes and several of them are known to play a significant role in the development of various cancers. In this initial study, we used a panel of both NSCLC and SCLC cell lines to determine the relative levels of various Pax proteins.

Methods: Whole cell lysates from a panel of NSCLC and SCLC cell lines were prepared using RIPA Buffer. Equivalent amount cell lysates proteins were separated by SDS-PAGE and subjected to immunoblotting using various commercially available anti-Pax antibodies.

Specific Pax knockdown cells were also generated by transfecting commercially available siRNA. The loss of the particular Pax expression was determined using immunoblotting procedures.

Results: We detected significant expression of Pax8 in NSCLC and Pax5 in SCLC cell lines. In addition we could detect BCL2 but not BCL-XL, especially in the SCLC cell lysates. Since Pax3 is a known direct transcriptional activator of c-Met, a receptor tyrosine kinase that is known to play a significant role in cancer metastasis, we therefore determined the protein expression levels of c-Met in the above cell lines. In general, in most of the cell lines where Pax expression was detected, we also detected comparable levels of c-MET. We are currently determining how the Pax factors can regulate biological and biochemical processes in lung cancer.

Conclusions: Differential expression of Pax8 in NSCLC and Pax5 in SCLC can be further tested as biomarkers to characterize lung tumor tissue biopsies. This differential expression can be combined with other biomarkers to help distinguish between SCLC, NSCLC, and other tumors.

P2-127 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Effects of insulin like growth factor-1 on repair mechanism of DNA damage induced by cis-Diammineplatinum dichloride in NSCLC

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Propose: Authors are to investigate DNA repair mechanisms involved in DNA damage induced by cis-Diammineplatinum dichloride(cisplatin), which is widely used for treatment of NSCLC. We also studied relevance of IGF-1 system in DNA repair mechanisms.

Methods: The effect on non-small cell lung cancer (NSCLC) cell line NCI-H1299 and NCI-H460 proliferation by IGF-1 and cisplatin treatment was measured by MTT assay. Changes of molecular system consisting HRR and NEJH were evaluated by immunoblotting and immunocytochemistry. Comet assay was applied to analyze influence of IGF-1 on DNA damage repair.

Results:

1. Cisplatin treatment resulted in inhibition of cell proliferation in a dose dependent manner. IC50 and IC80 are about 33.3 uM and 9.1 uM in H1299 and 33.7uM and 8uM in H460 cells. 50 ng/ml IGF-1 treatment on each cells recovered about 20% of cell proliferation repressed by cisplatin.
2. Immunocytochemical study showed cisplatin treatment induced activation of gamma H2AX, and addition of 50 ng/mL IGF-1 potentated its activation. Nuclear translocation of ATM and IRS-1 was promoted by cisplatin treatment, but was suppressed by IGF-1. On the other hand, translocation of ATR was enhanced by cisplatin and facilitated by IGF-1 treatment.
3. Phsphorylation of ATM induced by cisplatin was confirmed by immunoblotting, but not with IGF-1. Whereas IGF-1 induced ATR activation, and promoted gamma H2AX formation.
4. Reduced cisplatin induced DNA damage could verify IGF-1 effect with comet assay.

Conclusions: Activation of ATR pathway by IGF-1 might be primary recovery mechanism of cisplatin induced DNA damage.

P2-128 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Sequence dependant antiproliferative effect of cytotoxic drugs and epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) in non-small cell lung cancer cell lines (NCI-H1975).

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Introduction: EGFR-TIKs showed about twenty percent of response rate in refractory non-small cell lung cancer. Clinical trials of cytotoxic drugs and EGFR-TIKs failed to show improved survival compared to platinum based doublets. An antagonism between EGFR-TKIs and cytotoxic chemotherapy drugs was raised as a possible explanation for the negative results.

Materials and Methods: The antiproliferative effects and cell cycle distributions after treatments with EGFR-TKIs(gefitinib and erlotinib) and cytotoxic drugs(Docetaxel, Paclitaxel, Gemcitabine) were studied using a cell line(NCI-H1975, adenocarcinoma of lung) harboring T790M mutation in exon 20 of EGFR gene. The cell viability assay and cell cycle analysis were performed with MTT assay and flow cytometry. EGFR-TKIs and cytotoxic drugs were treated in different sequences to observe sequence dependent effect. Calcusyn software(Biosoft, Cambridge, UK) was used to calculate combination index(CI).

Results: Various combinations of cytotoxic drugs and EGFR-TKIs showed different antiproliferative effects on NCI-H1975 cell line. Antagonisms(CI>1) were observed when EGFR-TKIs were treated before cytotoxic drugs(EC sequence), while synergisms(CI<1) were observed when cytotoxic drugs were pre-treated before EGFR-TKIs(CE sequence). Treatment in EC sequence arrested the cells in G0/G1 phase and decreased the apoptotic fraction. However, treatment in CE sequence arrested the cells in G2/M phase and higher fractions of apoptotic cell death were observed.

Conclusion: To combine EGFR-TKIs and cytotoxic drugs, sequence dependent anti-proliferative effects should be considered.

P2-129 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Galectin-9 in stroma is a better prognostic indicator in lung cancer -Tissue Microarray Analysis

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Galectin-9 is a member of the β-galactoside-binding galectin family proteins associated with diverse biological processes, such as apoptosis, cell aggregation and eosinophilic chemoattraction. Some reports described that galectin-9 was a possible prognostic factor in breast cancer and melanoma.

We have found stromal spindle cells are occasionally positive for galectin-9 in our previous study (data not shown). Herein, we investigated its clinicopathological significance using lung cancer tissue microarray (TMA). We immunohistochemically examined the expression of galectin-9 in lung cancer using TMA containing samples from 400 surgical cases. Cancerous stroma was microscopically recognized in 183 cases (109 adenocarcinoma, 70 squamous cell carcinoma and 4 adenosquamous cell carcinoma cases).

Total of 24.6 % cases (45/183) showed galectin-9 expression in stromal spindle cells. We examined the survival statistical significance of galectin-9 using the log-rank test, and Kaplan-Meier curves were plotted. Positive immunohistochemical staining with galectin-9 was associated with favorer survival for patients with lung cancer (5-year survival of 59.2% versus 31.3% p=0.0206).

Conclusion: Our data indicates that galectin-9 in cancerous stroma can be a better prognostic biomarker in lung cancer.

P2-130 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Podoplanin expression in cancerous stroma is a poor prognostic marker- Tissue Microarray Analysis

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Podoplanin is a mucin-type glycoprotein and a noble lymphatic endothelial marker. Immunohistochemical staining against podoplanin is currently a useful tool to detect lymphatic involvement of cancer cells, and is widely used in a routine pathological diagnosis. By observation of daily cases, we have noticed stromal spindle cells are occasionally positive for podoplanin. To confirm its presence and to investigate its clinical significance, we immunohistochemically examined podoplanin expression using several monoclonal antibodies and tissue microarrays.

We found that stromal podoplanin expression in adenocarcinoma was significantly associated with poorer prognosis ($p<0.001$). The prognostic significance was still high after adjustment with stage, gender, age, and histological differentiation ($p<0.001$). The expression was associated with differentiation and tended to associate with nodal metastasis. Also we immunohistochemically examined with 14 common cancer types and found that podoplanin expression was significantly associated with nodal metastasis ($p<.01$). Our data indicates that podoplanin expression in cancerous stromal cells may play a critical role in lymphatic invasion of cancer cells to determine patients' survival.

P2-131 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Increased expression of survivin and its splice variants in non-small cell lung carcinoma

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Background: Apoptosome pathway dysfunction in tumor cells may account for their apoptosis resistance and there is evidence that survivin (Sur) and some its alternative splice variants may be involved in regulation of this cell death pathway in malignant tumors. In this work, we studied the expression of transcripts encoding Sur and its splice variants Sur-3B, Sur-2B and Sur-ΔEx3 in non-small cell lung carcinoma (NSCLC) tissues and lung parenchyma from surgically treated patients and examined the impact of survivin gene promoter genotype at nucleotide -31 and of the smoking status of NSCLC patients on the expression level of the indicated Sur transcript variants.

Methods: The expression of mRNAs encoding Sur, Sur-3B, Sur-2B and Sur-ΔEx3 was quantitated by real time RT-PCR using transcript-specific oligonucleotide primers and TaqMan fluorogenic probes, and an input of total RNA isolated from NSCLC and lung tissues. The expression of Sur transcript variants was normalized against the expression of β-actin mRNA. Genotyping of the survivin gene promoter in NSCLC and lung tissues was performed by PCR amplification and DNA sequencing of the purified PCR product with nested primers.