found to be significantly different between the two classes ($p<6.5E-6$, corrected for multiple testing). In addition, the two different tumor types can be classified with an accuracy of 74%. Our study describes a unique chip based technique for assessing aberrant gene methylation which was used to analyze 60 genes in 37 individuals with lung cancer and normal controls. The results provide compelling evidence that, based on their methylation patterns, different types of lung cancers and normal lung tissues can be distinguished with high accuracy and may prove have great potential in population based screening for lung cancer.

#5563 **Aberrant promoter methylation of insulin-like growth factor binding protein-3 is associated with poor clinical outcome in stage I non-small cell lung cancer.** Yoon Soo Chang, Luo Wang, Diane Liu, Fadlo Khuri, Li Mao, Jonathan M. Kurie, Waun K. I. Hong, and Ho-Young Lee. *M. D. Anderson Cancer Center, Houston, TX.*

Insulin-like growth factor (IGF) binding protein-3 (IGFBP-3) is known to block IGF action and functions as a cell growth inhibitor and/or promoter of apoptosis. In addition to its role in modulating IGF function, IGFBP-3 has direct IGF-independent effects on cellular function. Because IGFBP-3 expression is lost in several non-small cell lung cancer (NSCLC) cell lines, we investigated whether methylation of IGFBP-3 promoter might lead to silencing of IGFBP-3 gene in human lung tumor. Methods: 14 NSCLC and 8 SCLC cell lines and specimens from 90 patients who underwent curative surgery at M.D. Anderson Cancer Center under the diagnosis of stage I NSCLC were investigated for promoter methylation and methylation specific polymerase chain reaction (MSP) methods. Statistical analyses were preformed to evaluate clinical parameter associated with methylation status and to determine prognostic effect of aberrant methylation. Results: IGFBP-3 promoter was methylated in 50.0 % of NCSC (7 of 14) and in 37.5 % of SCLC cell line (3 of 8) and this phenomenon was corresponded with down-regulation of IGFBP-3 mRNA and protein level. Aberrant methylation of IGFBP-3 promoter was found in 64.4 % of patients who diagnosed stage I NSCLC (58 of 90). Patients with aberrant promoter methylation had a statistically significant poorer disease free survival and overall survival comparing those without aberrant methylation. In patients who were diagnosed as pathologic stage I only aberrant promoter methylation status was an independent factor that could predict recurrence/metastasis and poorer overall survival. Conclusion: aberrant methylation of the IGFBP-3 promoter could be one of mechanism that regulates expression of IGFBP-3. This abnormality is strongly associated with disease recurrence/metastases and poor clinical outcome, suggesting that IGFBP-3 play an important role in determining the biologic aggressiveness of NSCLC.

#5564 **p16 methylation in sputum samples from patients with non-small cell lung cancer.** Jeanne P. Harvey, Allison W. Gannon, Rick Rogers, and John F. Lechner. *Bayer Diagnostics, Berkeley, CA.*

The p16 (CDKN2/MTS-1/INK4A) tumor-suppressor protein is frequently absent in non-small cell lung cancer because of DNA methylation of CpG islands within the promoter and first exon of the gene. We have evaluated for the presence of p16 methylated sequences in a set of 69 induced sputum samples collected from individuals just prior to surgery for lung cancer and compared the data against a set of samples from 42 smokers with no evidence of disease, using methylation-specific PCR. Approximately one-third of the cancer patients had endobronchial lesions (visible on bronchoscopy) and two-thirds had peripheral lesions. All stages of disease were represented. Smokers had at least a 10 pack-year history of smoking. Of these 17 were heavy smokers with greater than 30 pack-years of smoking. The Saccomanno method was used for the collection, processing, and storage of the sputum samples. Sputum specimen adequacy was assessed microscopically after Papanicolaou staining to ensure that each specimen contained a sufficient number of cells of deep lung origin. Stability of DNA stored in Saccomanno's preservative was pre-verified by assessing cell lines and sputum samples after fixation at various time points up to a year. Both DNA yield and quality, as assessed by the ability to be PCR amplified, were thereby assured. Unmethylated p16 sequences were detected in all samples, reflecting the background of DNA from non-cancerous cells. Methylated p16 sequences were seen in 43% (9/21) of the central airway cancer specimens, 40% (19/48) of the peripheral lung cancer specimens, and 31% of the samples from smokers without cancer. No correlation was seen between pack-years smoked and p16 methylation. This study supports other reports in the literature that p16 methylation occurs frequently in the bronchial epithelium of NSCLC patients, as well as in smokers without cancer. Aberrant p16methylation has been shown to be present in even the earliest stages of respiratory carcinogenesis. However, since <15% of smokers are expected to develop lung cancer, in order to have diagnostic utility, a panel of markers would be required to provide requisite specificity. Accordingly, data for additional frequently methylated genes will also be presented.

#5566 **The candidate tumor suppressor gene hSRBC is frequently methylated in lung cancer.** Sabine Zöchbauer-Müller, Kwun M. Fong, Xie Xu, Joseph Geradts, Michael Peyton, Christoph C. Zielinski, Adi F. Gazdar, and John D. Minna. *University of Vienna Medical School, Vienna, Austria, The Prince Charles Hospital, Brisbane, Australia, University of Texas Southwestern Medical Center, Dallas, TX, and Roswell Park Cancer Institute, Buffalo, NY.*

Frequent allele loss in the chromosomal region 11p15.5-p15.4 has been described in various cancer types including lung cancer suggesting that this region harbors one or more tumor suppressor gene(s) (TSG). Recently, the human SRBC gene (hSRBC) which interacts with BRCA1 in a yeast two hybrid assay, has been mapped to this region and shown to undergo epigenetic inactivation in lung and breast cancer (Cancer Res 61: 7943, 2001). Western blot analysis on several lung cancer cell lines as well as immunostaining results on a few primary lung cancers demonstrated that loss of hSRBC protein expression occurs frequently in this tumor type. Aberrant methylation of the promoter region of TSGs has been identified as an alternative for the loss of gene function by deletion or mutation. Sodium bisulfite sequencing of the promoter region of hSRBC in several lung cancer cell lines which do not express this gene suggested that aberrant methylation plays an important role in inactivating hSRBC. To determine the methylation status of hSRBC in a large collection of primary lung cancer samples we designed primers for a methylation-specific PCR assay. Using this assay we investigated promoter region methylation of hSRBC in 107 resected non-small cell lung cancers (NSCLC) and their corresponding non-malignant lung tissues. This cohort has been previously studied for a large number of genetic and epigenetic markers and has detailed staging and survival data available (c.f. Cancer Res 61:249, 2001). Aberrant methylation was detected in 38% of resected NSCLCs, but was not seen in the majority of corresponding non-malignant lung tissues. The pattern of hSRBC methylation is being compared with loss of hSRBC protein expression by immunostaining using a monoclonal antibody. The methylation changes are being correlated with histology, stage, smoking history and survival of the patients. Our findings suggest that the BRCA1 interacting protein hSRBC is a candidate TSG which is frequently inactivated by aberrant promoter methylation in the pathogenesis of lung cancer.

#5567 **Alterations of methylation in DNA from human lung cancer tissues.** Nagalakshmi Keshava, Deborah Huffman, Zhong-Liang Wu, and Tong-man Ong. *National Institute for Occupational Safety and Health, Morgantown, WV, and Guangzhou Medical College, Guangzhou, China.*

Aberrant methylation pattern is an acquired epigenetic alteration causing inappropriate activation or silencing of a gene. Alterations in DNA methylation have been associated with cancers at almost all tumor sites and represent one of the most consistent changes in neoplastic cells. To determine if global methylation may contribute to the development of lung cancer, we studied genome-wide aberrant methylation pattern in 57 lung cancer cases and matched controls. Methylation was carried out using methylation sensitive restriction DNA fingerprinting analysis. We found that 86% of all the lung cancer tissues were hypermethylated at various sites using short random primers. Many fragments appeared to be differentially methylated. Upon sub-cloning, sequencing and matching several common differentially methylated fragments using the available database (BLAST), we have identified the fragments to encode for human cyclin C (CCNC), Wilms tumor (WT-1), Nuclear factor−κ B (NF-kB) genes. Analysis of fragments among tumor types revealed that 49% of the hypermethylated fragments were adenocarcinomas, 30% were squamous cell carcinomas and 21% belonged to other types. When age or sex was considered as a factor, no significant difference in any of these groups were observed. Methylation pattern was unrelated to smoking status of the patients. We also studied the methylation status of the tumor suppressor *p16* gene using bisulfide modification method. Hypermethylation in the *p16* gene was observed in 45/60 samples (75%). Our overall results indicate that hypermethylation seems to play an important role in the davelopment of lung cancer. Further studies are in progress to elucidate the molecular mechanism(s) of hypermethylation in lung cancer.

#5568 **Identification of genes responsible for demethylation-induced growth inhibition of human lung cancer cells.** Bao-Zhu Yuan and Steven Reynolds. *Genetic Susceptibility Laboratory, Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV.*

Evidence suggests that demethylation treatment of tumor cells by 5-aza-2'-deoxycytidine (5-aza-dC), an agent commonly used for re-inducing the expression of hypermethylation-silenced tumor suppressor genes, can induce cell growth inhibiton, apoptosis and cell differentiation. 5-aza-dC exerts its effects on tumor cells through the inhibition of DNA methyltransferase activity and the subsequent demethylation of DNA hypermethylation-silenced genes. In this study we initially determined the effect of 5-aza-dC on five human lung cancer cell lines and then attempted to identify the genes responsible for 5-aza-dC-induced effects by cDNA array analysis. It was found that the treatment of 1uM 5-aza-dC can induce growth inhibition in all five lung cancer cell lines. Further observations on NCI-H522, an adenocarcinoma cell line, showed that 1uM 5-aza-dC can induce cell cycle arrest in S phase and apoptosis as tested by Tunel assay. A cDNA array analysis covering 1176 genes (Clontech) were performed on 5-aza-dC treated and untreated NCI-H522 cells to identify the genes responsible for the induction of cell growth inhibition. It was found that 48 genes and 24 genes were down-regulated or up-regulated, respectively, by 1uM 5-aza-dC treatment. Among the down-regulated genes were membrane-associated protein (Myt-1), Dishevelled 1(DVL1) and Catalase B genes, while among up-regulated genes were Hint/PKC-I, S100 calcium-binding protein A4 (S100-A4), Tax1-binding protein 151 (TXBP151) and Chromatin assembly factor 1 genes. These results were confirmed by Northern blot and/or RT-PCR. Quantitative RT-PCR showed that the significant change of expression for Myt-1, DVL1, Hint/PCK-I and Chromatin assembly factor genes happened at 36 hours after 5-aza-dC treatment, which is in accordance with the time for 5-aza-dC to initiate the inhibition of DNA meth-