would help us to discriminate different NSCLC types and eventually predict the survival and clinical course of the patients.

Our previously performed studies with these tissue samples have shown upregulation of well-known cancer associated genes like STEAP and downregulation of DAPK1, TNFSF10 and EDG1.

### P2-031

**BSTB: Cancer Genetics Posters, Tue, Sept 4**

**Microarray gene expression in primary lung adenocarcinoma classified by lung asbestos burden**

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**Background:** Asbestos is recognised by the IARC as a human lung carcinogen. The relationship between asbestos and tobacco in lung cancer causation has proven difficult to define, and causal attribution of lung cancer in smokers who have also been exposed to asbestos is often difficult. There are no clinical or pathological characteristics which distinguish lung cancers partly or wholly attributable to asbestos from those due to tobacco alone. The hypothesis for this study was that, despite their phenotypic similarity, lung cancers arising in subjects exposed to respirable asbestos have a different gene expression profile from those arising in subjects with no asbestos exposure.

**Subjects and Methods:** With institutional ethics committee approval we performed MIAME compliant microarray expression analysis on RNA extracted from resected tumour tissue in 37 cases of adenocarcinoma (AC) on the Operon Human V.2 22k platform. Lung asbestos fibre burden was measured by tissue digestion and filtration, counting ferruginous bodies (FB) by light microscopy. Fibre burdens >20 FB per gram wet weight (g/ww) are usually associated with occupational or significant environmental exposure to respirable asbestos (1). Tumour gene expression was compared between 24 subjects with 0 FB/gww (Group 1) and 13 subjects with >=20 FB/gww (Group 2) using Avadis software. Subject groups were similar for age, gender, and smoking.

**Class prediction was performed in BRB Array Tools v3.5.**

**Results:**

1. Volcano plots identified 21 probes with significantly different expression at p=0.001 and magnitude of absolute fold change >1.5 between these two classes, almost all upregulated in Group 2. The probes corresponded to one pseudogene, ten unknown genes and ten annotated genes including genes in the RAS pathway, novel zinc finger proteins and genes with redox function. The direction of expression difference was validated for 5 out of 6 of these genes by RT-PCR in an independent test set of 30 adenocarcinomas.

2. A 95-gene classifier was developed using the 1 nearest neighbour prediction model by leave one out cross-validation. The mean correct classification rate was 89%, permutation p=0.002. Negative predictive value was 0.852 indicating potential of this classifier to rule out adenocarcinoma related to asbestos exposure. Receiver operating analysis showed that the top 8 genes (by p value) could generate over 90% of the performance of the full classifier.

**Conclusions:** AC in subjects with lung asbestos bodies showed significant upregulation of several genes compared with AC in individuals without lung asbestos. This finding was confirmed by an independent method in independent subjects, implicating these genes as possible players in asbestos carcinogenicity and / or tumour progression. A 95-gene set was predictive of AC class based upon lung asbestos fibre burden. The performance of an 8-gene subset requires independent evaluation for predictive utility as an RT-PCR panel in clinical and medico-legal settings.


### P2-032

**BSTB: Cancer Genetics Posters, Tue, Sept 4**

**Human Lung Cancer Related New Genes**

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Lung cancer is a leading cause of death worldwide. Lung cancer is becoming the major malignancy in China. Many events including alterations of oncogenes and tumor suppressor genes must have occurred by the time when lung cancer becomes clinically evident. We used the following methods including comparative genomic hybridization; alleotyping; cDNA library construction; and DNA microarray to investigate human lung cancer related genes. On the basis of the above mentioned study, we cloned more than 50 genes, which had never been deeply characterized before.

We identified a novel gene highly expressed in human lung cancer tissue that we named OLC (overexpressed in lung cancer). Forced overexpression of OLC malignantly transformed NIH3T3 cells in vitro and in vivo. Immumohistochemistry staining indicated that OLC was overexpressed in human lung dysplasia/carcinoma in situ and non-small cell lung cancer. The overexpression of OLC was also observed in human esophageal and laryngeal carcinoma. Small interfering RNAs (siRNA) mediated OLC gene silencing in two lung cancer cell lines (H520, H1299) which highly expressed OLC protein, induced significant reduction of cellular growth and high rates of apoptosis. Using an EMSA supershift assay we demonstrated that OLC overexpression can induce translocation of the NF-kappaB complex (p50/p65) from cytoplasm to nucleus in Hela, H520, and H1299 cancer cells. Our preliminary data showed a higher OLC expression in lung squamous cell carcinoma (SCC) of smoker patients than in lung SCC of non-smokers, and cigarette smoke condensate treatment increased OLC expression in human bronchial epithelial cells in vitro. This study indicates that elevation of OLC might be associated with cigarette smoking-related human lung carcinogenesis, particularly at early stage.

Another gene, nominated DENND2D by HGNC, from the novel gene pool constructed in our laboratory was investigated. DENND2D suppressed the malignant transformation of NIH/3T3 cells. Transfection of DENND2D gene into the non-small cell lung cancer cell line, H1299, inhibited the cell proliferation and anchorage-independent growth in vitro, and reduced tumorigenicity of H1299 cell in nude mice. Flow cytometry assay revealed that expression of DENND2D induced G1/S-phase arrest in the cells. Semi-quantitative reverse transcription-PCR demonstrated that down-regulated expression of DENND2D was observed in lung cancer tissue samples, lung cancer cell lines, and the immortalized human bronchial epithelial cell lines, whereas DENND2D remains its expression in the primarily cultured normal bronchial epithelial cells. The lung cancer related novel gene DENND2D may play important role(s) in the pathogenesis of human lung cancers.