**LKB1** gene mutations are infrequent in Japanese patients with lung cancer

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**Background:** *LKB1* (also known as *STK11*; locus 19p13.3) is regarded as a causative gene of Peutz-Jeghers syndrome. Somatic mutations in the *LKB1* gene are relatively rare in sporadic cancers, but they are reported to occur in 26% of lung adenocarcinomas in Caucasian patients and in 54% of lung adenocarcinoma cell lines. The aim of this study was to determine mutational frequency of the *LKB1* gene and to investigate the expression levels of the *LKB1* gene in Japanese patients with lung cancer.

**Methods:** We sequenced the *LKB1* gene in 22 lung cancer cell lines (including eight adenocarcinomas, six squamous cell carcinomas, three large-cell carcinomas, and five small-cell carcinomas; eleven were of Japanese origin and the others were of Caucasian origin). One-hundred resected specimens (81 adenocarcinomas, 14 squamous cell carcinomas, and five other histological types) from Japanese patients with primary lung cancer were also analyzed. In addition, the expression levels of the *LKB1* gene were determined by quantitative real-time reverse transcription-PCR and the results were correlated with the clinical and pathological features of patients including *KRAS*, *TP53*, *EGFR*, and *HER2* gene status.

**Results:** Among the 22 cell lines, four had *LKB1* mutations. The mutations detected were Gln to stop at codon 37 (A549, NCI-H460), Trp to stop at codon 332 (H23), and a deletion of codons 465-97 (VMRC-LCD). They were all Caucasian origin, and three of them were in adenocarcinoma. In 100 resected lung cancer specimens, only three patients had *LKB1* somatic mutations (3%). One was a one-base deletion mutation (842delC), and two were missense mutations (Pro to Leu at codon 281[P281L], and Trp to Leu at codon 308[W308L]). W308L has not been reported previously. All patients with the *LKB1* gene mutation were male smokers with adenocarcinomas. Of these three cases, two had poorly differentiated tumors and one had well differentiated tumor. Two were stage IIIA and one was stage IB. These three patients each had another mutation in either of the *EGFR*, *KRAS* or *TP53* gene. No significant correlation was observed between the expression level of *LKB1* and clinicopathological features.

**Conclusions:** *LKB1* gene mutations appear to be relatively rare in Japanese patients with lung cancer compared with Caucasian patients. Tumors with *LKB1* mutation seem to have high malignant potential. However, *LKB1* seems to play a minor role in the lung cancer of Japanese patients, because of low tendency of *LKB1* mutations in Japanese.

**Blood lymphocyte cytochrome P4501A1: A biomarker to predict lung cancer**

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**Background:** Worldwide, lung cancer continues to be the leading cause of cancer death in both men and women. Tobacco smoking remains the single most important risk factor for development of lung cancer, especially in males. Cytochrome P450 1A (CYP1A1) family has been shown to play an important role in the metabolic activation of the constituents of tobacco smoke and other polycyclic aromatic hydrocarbons (PAHs). CYP1A inducibility has been shown to be a susceptibility biomarker for lung cancer. Several studies have demonstrated an association between CYP1A-dependent enzyme inducibility and tobacco induced lung cancer risk. As CYP1A1 has been shown to be expressed in blood lymphocytes, blood CYP1A profile could be potential candidate for use as a biomarker to predict PAH induced toxicity including lung cancer. The present study attempted to utilize blood lymphocyte CYP1A1 expression profiles as a possible biomarker for lung cancer by studying the CYP1A1 mRNA expression and the activity of 7-ethoxyresorufin-O-deethylase (EROD) in the blood lymphocytes of patients suffering from lung cancer, prior to the treatment (chemo/radiotherapy) and after the treatment during follow-up.

**Methods:** Lung cancer patients visiting OPD facility of Department of Radiotherapy of King George’s Medical University, Lucknow and age and sex matched controls were included in the study. Blood was drawn from the patients prior to the start of the treatment and after treatment (routine regimen of chemotherapy and radiotherapy) was completed. Blood was processed for the isolation of lymphocytes and the activity of EROD was assayed in the blood lymphocytes. Total RNA was extracted from whole blood with TRI-BD (Sigma-USA) according to manufacturer’s protocol and was analyzed for CYP1A1 mRNA by RT-PCR analysis.

**Results:** RT-PCR of mRNA collected from blood lymphocytes isolated from controls and patients, after normalization with β-actin, an housekeeping gene revealed significant increase in the mRNA expression of CYP1A1 in the patients (103%) suffering from squamous cell carcinoma of lung malignancy. The increase in blood lymphocyte CYP1A1 mRNA was associated with significant increase (179%) in the activity of EROD in blood lymphocytes isolated from patients suffering from lung cancer. After receiving standard dose of chemotherapy and radiotherapy, the increase in the blood lymphocyte CYP1A1 mRNA levels in the patients was found to decline (20% increase), though the CYP1A1 expression was found to be higher than the controls. Our data further revealed that associated with the increase in mRNA expression, a significant increase in the activity of CYP1A1-dependent EROD (179%) was observed in the blood lymphocytes of patients suffering from lung cancer. Treatment (chemotherapy and radiotherapy) resulted in the decline in the induced enzyme levels, though the activity remained elevated (100%) when compared to the controls.

**Conclusion:** Increase in the blood lymphocyte CYP1A1 levels in the lung cancer patients and a trend towards the decline in the induced mRNA levels of CYP1A1 following chemotherapy and radiotherapy.
have demonstrated that blood lymphocyte CYP1A1 could be used a biomarker to predict lung cancer.

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Promoter analysis of co-expressing genes after exposure to asbestos
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Background: Occupational exposure to asbestos is associated with the development of asbestosis, malignant mesothelioma, and lung cancer. Research related to the mechanisms by which asbestos fibers cause damage in the cells and which are the mediator genes is important as exact mechanisms underlying asbestos-associated carcinogenesis are not known. Knowledge about the genome-wide alterations involved in asbestos-associated carcinogenesis can be obtained by characterizing changes in gene expression after exposure to the carcinogen or between asbestos-exposed and non-exposed patients using microarrays.

Methods: We have previously analyzed the DNA copy number and gene expression profiles of 14 lung tumors from highly asbestos-exposed and 14 non-exposed patients using microarrays and revealed that a specific aberration profile could be characteristic of lung tumors associated with asbestos-exposure. Furthermore, by exposing human epithelial and mesothelial cells to crocidolite asbestos and performing time-series microarray experiments, we have recently reported asbestos-exposure related temporal changes in gene expression profiles. In this study, the gene expression microarray data from these experiments were combined. Promoter sequence analysis was first applied to clusters of co-expressed genes detected from the cell line data to identify genes that are likely co-regulated by the same mechanisms. Thereafter, common changes between the cell line and patient data were revealed.

Results: 15 transcription factors were identified to be under-represented in the promoter regions of genes with similar temporal expression profiles in comparison to the remaining genes on the array (p<0.00001). Among the under-represented transcription factors were those that have been previously associated with regulation of mitochondria, oxidative stress, and carcinogenesis but not with exposure to asbestos.

Conclusions: The study provides new information about putative transcription factors and mediator genes involved in asbestos-associated carcinogenesis. We show that the integration of gene expression data from cell line and human studies gives insight of the mechanisms underlying asbestos-related carcinogenesis.

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Association of cytochrome P450 polymorphism and its combination genotypes with lung cancer risk
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Background: Globally, lung cancer is the most frequent cancer today and is expected to have a major impact on human health throughout the next decades. The role of tobacco smoking as a major etiologic factor of this malignancy is well established. The gene-environment interaction for cancer development is largely attributed to the action of xenobiotic metabolizing enzymes (XME). Genetic polymorphism is known for the xenobiotic metabolizing cytochrome P450 (CYPs), the phase I enzymes and glutathione-S-transferases (GST), the phase II enzymes, involved in the process of carcinogenesis. As not much information is available on the polymorphism of CYP and GST isoenzymes in Indian population, the present case-control study attempted to investigate the association of polymorphism in CYP1A1, 1B1 and GSTM1 and the combination of these important polymorphisms with squamous cell carcinoma of lung malignancy.

Methods: Patients suffering from squamous cell carcinoma of lung (n=140) and visiting OPD facility of Department of Radiotherapy, King George’s Medical University, Lucknow, India were included in the study. Equal number of age- and sex matched healthy individuals were also enrolled in the study. After obtaining detailed information from each individual, 1.0 ml blood was drawn from them which were processed for isolation of DNA. PCR amplification were carried for studying the polymorphisms in CYP1A1, CYP1B1 and GSTM1 using the standardized protocols.

Results: Our data revealed that the variant alleles of CYP1A1 (both,Msp1, Ile/ Val and BSaI) were found to be significantly overrepresented in the lung cancer patients when compared with the controls. Haplotype analysis revealed that haplotype, C-A-C and A-A-T were associated with significant increase in the lung cancer risk. As observed with CYP1A1, the frequency of the variant genotype of CYP1B1 (Arg48Gly/ Ala119Ser and Leu432Val) were significantly increased in the patients resulting in significant increase in the lung cancer risk. Haplotypes having 48arginine, 119alanine, 432leucine and 453serine or 48glycine, 119serine, 432valine and 453aspartigeine were found to be more prevalent in the cases and were associated with significant increase in the lung cancer risk. Cigarette smoking and tobacco chewing was further found to increase the risk in the cases. The genotype combinations of CYP1A1 and CYP1B1 increased several folds (6-8 folds) the risk associated with lung cancer. Likewise, combination of variant genotypes of CYP1A1 with null genotype of GSTM1 also increased the risk in the cases.

Conclusion: Our case-control study have shown that polymorphism in CYP1A1 and CYP1B1 may modify the risk to lung cancer. Haplotype approach revealed an stronger association of CYP1A1 or 1B1 haplotypes with risk to lung cancer. The increase in risk in cases was further found to be increased in cases carrying combination of variant genotypes of CYP1A1 and 1B1 and GSTM1. Furthermore several fold increase in the risk in smokers and tobacco chewers have demonstrated the importance of gene-environment interactions in the pathogenesis of lung cancer.