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Oligonucleotide Microarray Analysis of Lung Adenocarcinoma in Smokers and Nonsmokers Identifies GPC3 as a Potential Lung Tumor Suppressor*

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To investigate gene expression profiles associated with cigarette smoking and lung carcinogenesis, we obtained specimens of paired tumor and healthy lung tissue from six nonsmoking individuals who had lung carcinoma and from matched lung cancer patients with a history of smoking. Gene expression analysis was performed using an oligonucleotide array (HuGeneFL Array; Affymetrix, Santa Clara, CA). Hierarchical clustering demonstrated that healthy tissue and tumor tissue clustered separately and that within healthy tissues, those from smokers and nonsmokers clustered separately. Paired t tests were used to create gene lists for probes that demonstrated mean absolute differences (p < 0.01) in samples from tumors compared to healthy tissue for both smokers and nonsmokers. An interrogation of gene lists identified GPC3 as one of several genes the expression of which was lower in the healthy lung tissue of smokers than in nonsmokers and was lower in tumor tissue than in healthy tissue (p < 0.05). GPC3 encodes glypicans 3, a glycosylphosphatidylinositol-linked heparan sulfate proteoglycan. GPC3 alterations are associated with overgrowth in humans (Simpson-Golabi-Behmel syndrome) and in GPC3 null mice. Microarray results have been validated in human lung tissues with RNAase protection and with analysis of independent samples (U95Av2 array; Affymetrix). Northern blot analysis demonstrated that GPC3 expression was absent in 9 of 10 lung cancer cell lines. Ecotropic expression of rat GPC3 complementary DNA in pM-SCV-ires-GFP infected human lung carcinoma cells (A549 and NCI-H460 cell lines) suggests an association with growth inhibition in vitro. These results are being confirmed by an analysis of tumor formation by these

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cells in nude mice. In summary, these data suggest that GPC3 is a candidate lung tumor suppressor gene, the expression of which may be regulated by tobacco exposure.

**Differential Gene Expression of sFRP-1 and Apoptosis in Pulmonary Emphysema**

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*(CHEST 2002; 121:7S)*

In order to identify the unique molecular pathways that are involved in emphysema, a differential display analysis was performed on lung tissue to identify the genes expressed in the lung tissue of emphysema patients but not in that of patients with healthy lungs. We detected 142 differentially expressed genes. One of these genes, secreted frizzled-related protein sFRP-1, an inhibitor of Wnt signaling, was further characterized by its role in the pathophysiology of emphysema. Although this gene was not detected in the healthy adult mouse lung, it was expressed in the distal epithelial cells of the lung during development and also in the lung in two separate mouse emphysema models. Since the Wnt pathway is involved in proliferation and apoptosis, a study was undertaken that determined that tissue from the emphysematous lung of human patients was undergoing apoptosis. The apoptotic index closely correlated with the severity of disease. In *vitro* tissue transfection studies have confirmed that sFRP-1 leads to apoptosis of pulmonary epithelial and endothelial cells. The identification of sFRP-1 in emphysema has provided us with novel insight into the pathophysiology of this disease. The characterization of the other 142 differentially expressed genes will allow us to further identify the changes in gene expression in this disease. These genes have been placed on array chips so as to compare the differential gene expression from patients at various clinical stages of COPD with that of smokers prior to the development of COPD.

**Asthma Genetics**

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Asthma is the most common chronic childhood disease in developed nations and is a complex disease that has high social and economic costs. Asthma and its associated intermediate phenotypes are under a substantial degree of genetic control. Identifying the genes underlying asthma offers a means of better understanding its pathogenesis, with the promise of improving preventive strategies, diagnostic tools, and therapies. A number of chromosomal regions containing genes influencing asthma and atopy have been identified consistently by different groups, and a role for several candidate genes has been established.

*(CHEST 2002; 121:7S–13S)*

**Abbreviations:** HDM = house dust mite; IL = interleukin; LD = linkage disequilibrium; LPS = lipopolysaccharide; MHC = major histocompatibility complex; TCR = T-cell receptor; Th = T-helper cell; TNF = tumor necrosis factor

**Asthma** has become an epidemic, affecting 155 million individuals in the world. One child in seven in the United Kingdom wheezes, and similar numbers suffer from the related disorder of eczema (atopic dermatitis). Asthma is due to a combination of strong genetic and environmental factors. It has risen in prevalence over the past 30 years in all Westernized societies, perhaps as a result of the loss of childhood infections.

Many candidate gene and positional cloning studies of asthma have now been carried out. Although the number of candidate gene studies in asthma is growing rapidly, many contain small numbers of subjects and give equivocal results that do not subsequently replicate. This review will, therefore, concentrate on regions identified consistently through genetic linkage, because these by and large represent the strongest genetic effects, and candidates studied within these regions will be discussed in detail.

**Genome Screens**

The first genome-wide screen for linkages to quantitative traits underlying asthma identified significant evidence for linkage on chromosomes 4q, 6 (near the major histocompatibility complex [MHC]), 7, 11q (containing FceRI-β), 13q and 16. A replication sample of families in the same study confirmed linkage to chromosomes 4, 11, 13, and 16. A two-stage screen in Hutterite families from the United States found suggestive evidence for linkage and replication for loci on chromosome 5q, 12q, 19q, and 21q. A screen in German families identified suggestive evidence for linkage on chromosomes 4q, 6 (near the major histocompatibility complex [MHC]), 7, 11q (containing FceRI-β), 13q and 16.