STPOST 05

Comparison of gene expression profiles of cigarette smoke-exposed normal human bronchial epithelial (NHBE) cells to profiles from smokers and patients with chronic obstructive pulmonary disease (COPD) and lung cancer

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Abstract

Molecular changes in multiple signaling pathways that regulate oxidative stress, inflammation and xenobiotic metabolism are frequently observed in diseased lung. The purposes of this study were: 1) to assess differential gene expression profiles in human lung tissue or cells of non-smokers and smokers with and without lung cancer or COPD and 2) to compare gene expression changes induced by smoke in *in vitro* cultures of NHBE cells to expression profiles from *ex vivo* lung tissue samples.

Gene expression profiles were evaluated with the Affymetrix HG_U133 Plus 2.0 GeneChip Microarray. First, expression profiles which distinguished normal tissues from non-smokers and smokers were represented by 115 statistically significant probe sets (genes). Pathway and gene ontology analyses identified several ontologies containing overrepresented genes in smokers, including those involved in immune response and T cell activation. Second, gene profiles (7880 probe sets) and molecular pathways that distinguished malignant lung of smokers from the paired normal tissue were associated with immune response, cell cycle regulation and tryptophan metabolism. Third, comparisons of bronchial brushings from non-smoker, smoker and COPD (GOLD 0 – II) yielded 63 unique probe sets. NADPH quinone oxidoreductase (NQO1), mucin 5AC and glutamate decarboxylase 1 were among notable targets, and the top pathways represented oxidative stress, xenobiotic metabolism and hypoxia.

Finally, as we previously reported, the top responders in the NHBE cells exposed to repeated applications of cigarette smoke total particulate matter (TPM) were represented by 241 consistently regulated probe sets with significant deregulation of genes involved in oxidative stress, xenobiotic and tryptophan metabolism. Bioinformatic analyses were further employed to identify correlations with smoke and disease-impact in *ex vivo* lung samples. The gene profiles that were significantly modified in both CSC-exposed NHBE cells and COPD lung brushings included aldo-keto reductases (AKR1B10, AKR1C1, AKR1C2), cytochrome P450 1B1 (CYP1B1), NQO1, glutathione peroxidase 2 (GPX2) and SLC7A11, an amino acid transporter. Probe sets significantly regulated in both CSC-exposed NHBE cells and the cancerous and smoking-exposed lung were osteopontin (OPN/Spp1), dedicator of cytokinesis 10 (DOCK10), glycerol kinase (GK), kynureninase (KYNU) and transforming growth factor beta 2 (TGFB2).

Collectively, the data suggest that smoke-induced expression profiles in *in vitro* lung cell models are phenotypically relevant to lung modifications *in vivo* and may serve as potential biomarkers of effect.

Introduction

Many diseases, such as cancer, are associated with multiple alterations in genes and their expression. Chemical exposure modulated by inherited familial genetic polymorphisms drive such molecular genetic changes during the pathogenesis of disease. Gene expression profiles for inflammatory responses, oxidative stress and cellular proliferation are frequently altered in lung cells following exposure to airborne environmental mutagens and irritants and are associated with lung diseases such as chronic obstructive pulmonary disease, emphysema and cancer (Powell et al., 2003; Merkel et al., 2005; Shah et al., 2005; Spira et al., 2004). Gene expression analyses of lung biopsies, bronchial alveolar lavage and bronchial brushings are increasingly used to identify potential biomarkers (i.e. miRNA, DNA, mRNA, proteins) of smoking-related lung pathophysiological changes.

Mainstream cigarette smoke (MSS) is a complex chemical mixture characteristic of agents associated with the aggravation or alteration of normal cellular functions. The purposes of this study were: 1) to assess differential gene expression profiles in human lung (tissue or cells) from non-smokers and smokers with and without lung cancer or COPD and 2) to compare gene expression changes induced by smoke in in vitro cultures of normal human bronchial epithelial (NHBE) cells to expression profiles from ex vivo lung tissue samples.

Table 1. Gene Correlations Between Tissues and NHBE Cells

Source of Tissue/Cells	Number of Significant Probe Sets	Probe Sets Overlapping with NHBE-TPM	Top Pathways
Lung - Smoker/Cancerous	115	OPN/Spp1, DOCK10, GK, KYNU, TGFB2	Immune Response Cell Cycle Regulation Tryptophan Metabolism T-cell Activation
COPD Brushings	63	AKR1B10, AKR1C1, AKR1C2, CYP1B1, NQO1, GPX2, SLC7A11	Oxidative Stress Xenobiotic Metabolism Hypoxia

Table 2. Putative Biomarker Effects in NHBE Cells

Gene Symbol	Gene Name	Fold Change: CSC vs. DMSO						
		15min	30min	1h	4h	8h	12h	24h
KYNU	kynureninase (L-kynurenine hydrolase)				1.51	2.15	2.93	3.31
HMOX1	heme oxygenase (decycling) 1	-2.04	-1.91	1.61	47.13	48.33	81.81	4.93
SPP1	secreted phosphoprotein 1 (osteopontin)				3.35	6.79	10.21	12.76
CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1				4.64	3.96	5.24	7.92
NQO1	NAD(P)H dehydrogenase, quinone 1				1.84	2.52	3.49	3.58
GPX2	glutathione peroxidase 2 (gastrointestinal)				1.89	3.14	3.55	2.13

Results

Figure 1. Heatmap (A) and Representative Top Ten Responder (B) Non-Smoker vs. Smoker Lung Tissue

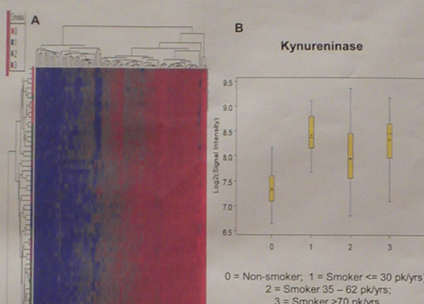


Figure 2. Heatmap (A) and Representative Top Ten Responder (B) Normal vs. Malignant Lung Tissue

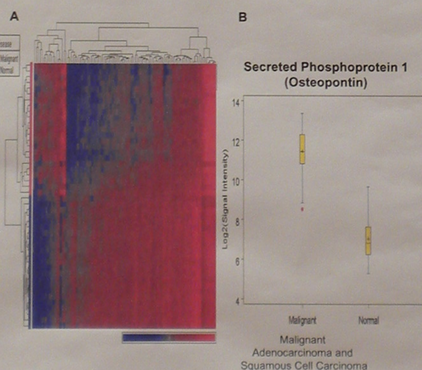
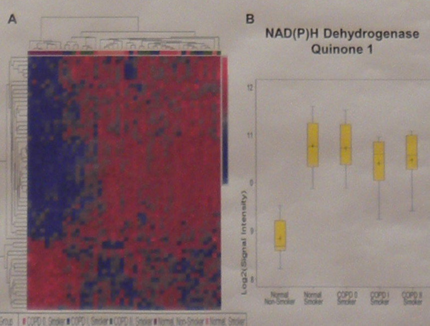


Figure 3. Heatmap (A) and Representative Top Ten Responder (B) Non-Smoker, Smoker and COPD



Materials and Methods

Cell Culture: NHBE donor lung cells were obtained from Lonza (Walkersville, MD), maintained in humidified incubators at 37°C and 5% CO2 and cultured in a modified LHC-9 medium (Lonza, Inc.; Walkersville, MD) as previously described (Fields et al., 2005). The donor was a non-smoking and disease free male.

Cigarette total particulate matter (TPM) Exposure: Kentucky Reference 3R4F cigarettes were used in this study. The TPM was prepared as previously described (Fields et al., 2005). TPM was collected from MSS onto a Cambridge filter pad and extracted with dimethylsulfoxide (DMSO) to yield a 30 mg TPM/ml stock solution. Cells were exposed to 3, 10, 30, 60 or 90 µg TPM/ml or DMSO (0.3%) in growth media continuously for 3, 6 or 24 hours. At each harvest, exposure media was removed, cells were lysed with TRIzol™ reagent (Invitrogen, Gaithersburg, MD) and stored at −80°C until RNA isolation.

RNA Isolation: Total RNA was extracted using TRIzol™ reagent according to the manufacturer's protocol. RNA quality and quantity were determined by Nanodrop and BioAnalyzer analyses, respectively.

Microarray Analysis: Gene expression profiles were generated with Affymetrix HG_U133 Plus 2.0 Gene Chip. Quality Control (QC) parameters (percent present, scale factor, etc.). Subsequently, differentially expressed probe sets were selected following statistical analyses (i.e. ANOVA, Dunnett's test, Benjamni & Hochberg False Discovery Rate) using the criteria of a corrected p-value < 0.05 and fold change > 1.5. Differential effects of the significant probe sets were represented box and whisker plots and heatmaps. Pathway and Gene Ontology (GO) analyses were conducted. All analyses were conducted by Gene Logic, Inc.

The analysis plan was conducted in four parts as detailed below.

Part I is a comparison of 58 pathologically normal lung tissues from non-smokers to similar tissue from smokers. Tissue samples (Gene Logic BioExpress®) are normal by pathology review; however, they come from patients who have neoplasms of the lung in adjacent areas. The lung tissues were separated into four smoking levels: non-smokers, light-smokers (≤ 30 pk. yrs.), medium-smokers (35 – 62 pk. yrs.), and heavy-smokers (>70 pk. yrs.).

Part II involved a total of 58 samples representing three disease status (normal, adenocarcinoma and squamous cell carcinoma) and four smoking levels [non-smokers, light-smokers (≤ 30 pk. yrs.), medium-smokers (35 – 62 pk. yrs.), and heavy-smokers (>70 pk. yrs.)]. Malignant denotes adenocarcinoma and squamous cell carcinoma.

Part III of the study involved the comparison of bronchial brushings from normal, COPD (Gold 0 - II), smokers and non-smokers. A total of 44 samples were used from the Gene Logic BioExpress® content.

Part IV: The results obtained from parts I - III were compared with those from previous work on gene expression in cultured bronchial epithelial cells exposed to TPM which yielded 241 stably-regulated probe sets. Comparisons of the significantly regulated gene profiles from each study were conducted as follows. The 241 stably-regulated probe sets from NHBE cells exposed to TPM were compared to 1) 115 significant probe sets from non-smokers vs. smoking history (separated by pack-years) from adjacent normal lung tissue of carcinoma donors; and 2) 63 significant probe sets from COPD vs normal lung brushing cell study.

Summary

- Pathway and gene ontology analyses identified several ontologies for the 115 probe sets that were overrepresented in smokers, including those involved in immune response and T cell activation. Kynureninase is a representative top responder that is associated with inflammation and cancer (Figures 1A, 1B and Table 1).
- Gene profiles and molecular pathways that distinguished malignant lung of smokers from the paired normal tissue were associated with immune response, cell cycle regulation and tryptophan metabolism. For example, osteopontin, a representative top responder, is a secretory protein involved in inflammatory response and elevated levels are observed in tumor development (Figures 2A, 2B and Table 1).
- NADPH quinone oxidoreductase (NQO1), mucin 5AC and glutamate decarboxylase 1 were among notable targets of the 63 probe sets showing differential regulation in bronchial brushings from non-smokers, smokers and COPD patients. The top pathways represented oxidative stress, xenobiotic metabolism and hypoxia (Figures 3A, 3B and Table 1).
- Gene profiles that were significantly modified in both TPM-exposed NHBE cells and COPD lung brushings included aldo-keto reductases, CYP1B1, NQO1, GPX2 and SLC7A11. Probe sets significantly regulated in both CSC-exposed NHBE cells and the cancerous and smoking-exposed lung were OPN/Spp1, DOCK10, GK, KYNU and TGFB2 (Tables 1 and 2).
- The data suggest that smoke-induced expression profiles in *in vitro* lung cell models are phenotypically relevant to lung modifications *in vivo* and may serve as potential biomarkers of effect.

References

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