Heterologous Gene Expression Profiling of Acute Lung Injury (ALI) Models: Search for the ALI Candidates Genes

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Rationale: We linked gene expression profiles (GEPs) of several *in vivo* (rodent, canine) and *in vitro* (human endothelial cell cultures) models of ALI by gene probe heterology into one database. A common heterologous gene expression trend across models identifies candidate genes involved in ALI response. **Methods:** GEPs were obtained from Affymetrix GeneChips: U34A for rat (n_{ctrl}=2, n_{ALI}=2), U74A for mouse (n_c=2, n_A=1), U133A for dog (n_c=4, n_A=4) and U95A for cultured endothelial cells (n_c=2, n_A=2). Probes from different GeneChips were grouped based on their heterology. The U95A had 10,507 homologs (>98% sequence identity) with the U133A and U74A, and U34A contained 5,134 and 1,990 heterologs with U133A (>86% sequence identity), respectively. Each heterolog-linked group was analyzed for consistency in gene expression (unidirection), amplitude of probe signal (>100 intensity units) and its significance ($p<0.06$, marginal call). **Results:** This algorithm selected 221 genes including several known ALI related genes: IL-6 (average change in gene expression across species +431%), MIF (+87%), IL-1Ra (+140%), which justified our approach. The analysis also revealed genes not previously associated with ALI: Ca/CAM-dependent PK-IV (+342%), IL-15 (-517%), FABP-6 (-239%), Tc. **Conclusions:** These data suggest n could be a useful tool in selecting cesses of interest. The overlapping s species strongly suggests an evolu- injury.

NHLBI HopGene PGA HC 66618

Candidate Surrogate Markers for COPD Identified by Differential Gen Expression

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by slowl progressive and irreversible airflow obstruction. Diagnosis is made followin spirometry, and often occurs at an advanced stage of the disease. FEV1 is less effec tive as a diagnostic of early phase disease. FEV1 is also used as a clinical endpoin to assess efficacy of novel drugs, using both acute changes and rate of decline However, due to the nature of the disease, such clinical trials have been long an expensive. Thus, development of accurate and accessible short-term surrogat markers of both disease progression and drug efficacy is essential to the develop ment of new medicines in COPD. We have identify candidate surrogate markers i the blood of extensively characterized (see table below) groups of COPD patients healthy smokers and healthy non-smokers. Total blood RNA was analysed for gen expression patterns using Affymetrix gene arrays U95Av2. Analysis of the gen expression data using Rosetta TM and SAS softwares, highlights subsets of diffe entially expressed genes among the three groups. A more precise analysis including steroid use and sputum production influences, has been used to refine th COPD-specific gene list.

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Early Gene Changes in a Mouse Alveolar Macrophage Cell Line in Response to Beryllium

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RATIONALE: Chronic Beryllium Disease (CBD) is an occupationally acquired lung disease that occurs as a cell mediated immune response to beryllium particles, resulting in the development of noncaseating granulomas. We hypothesized that the identification of early genes associated with beryllium exposure may give important insights into the role of macrophages in CBD. METHODS: We investigated multiple gene expression in a mouse alveolar macrophage (MH-S) cell line incubated in the presence or absence of 10μM $BeSO_4$ for 4 hours. We assessed cell viability following 4 hour incubation with beryllium and we determined that the cells were 95% viable and the proliferation rate matched those of unstimulated controls. We utilized Affymetrix oligonucleotide arrays consisting of approximately 12000 genes to detect differential changes in gene expression in beryllium-treated cells compared to controls. RESULTS: We found that beryllium caused significant changes in the expression of numerous genes encoding; heat shock proteins, cytokines, adhesion molecules, cytokine receptors, signalling molecules and transcriptional activators and repressors. Strikingly, heat shock protein and MHC class III region were elevated 10-fold in $BeSO_4$ treated macrophages. We verified that HSP70 mRNA was up-regulated over 10-fold by real time quantification (Taqman ™ PCR). The MHC class III region encodes heat shock proteins of the 70kDa family. HSP70 molecules play a critical role in cytoprotection against toxic exposures and are important as molecular chaperones in antigen processing and presentation. CONCLUSION: Our studies identify beryllium-regulated genes and suggest that HSP70, in particular, may play a pivotal role in cytoprotection and the early immune response to beryllium salts.

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Identifying Molecular Targets for Anti-Fibrotic Drug Discovery

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Evasion of apoptosis is a hallmark of diseases such as cancer and idiopathi pulmonary fibrosis (IPF). Apoptosis is regulated at all levels of control, including translational. When the activity of eukaryotic translation initiation factor 4E (eIF4E) is aberrantly increased, apoptosis is suppressed. In this regard, fibroblast cell lines derived from patients with IPF have pathologically elevated levels of eIF4E. We hypothesized that translationally regulated transcripts encoding rescue proteins would undergo increased translation initiation under proapoptotic conditions resulting in a greater number of bound ribosomes per transcript. As a model system, we compared NIH-3T3 fibroblasts to NIH-3T3 cells rendered resistant to apoptosis by stable overexpression of human eIF4E (3T3/4E). RNA isolated from cells in the basal state and under proapoptotic conditions was separated based on translational activity using sucrose density centrifugation, and the resultant fractions were subjected to microarray analysis. Here we present microarray data from two experiments using different gene arrays, identifying 8 to 12 candidate gene products. Real time PCR analysis of selected transcripts across the sucrose density confirmed the microarray data. Knock down experiments using inhibitory RNA to examine the effects of the identified genes on cell viability are in progress. We conclude that this method can identify transcripts that are subject to selective translational regulation in the context of fibroblast rescue from apoptosis by eIF4E, and suggest that this may be a powerful method to identify mRNAs encoding translationally regulated rescue proteins that are potential molecular targets for therapeutic intervention.

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Microarray Gene Expression Analysis Using Universal Reference RNA as a Standard

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RATIONALE. Using a common reference RNA as a standard in two-color microarray experiments provides reliable data comparison within and between experiments. Co-hybridization of Cy3-labeled common reference and Cy5-labeled experimental sample to the same microarray allows calculation of Cy3/Cy5 ratio at each probe position and normalization of the hybridization signal, thus significantly reducing variation in experimental conditions. METHODS. Total RNA from10 human, 11 mouse and 14 rat cell lines were pooled to make Universal Human Reference RNA (UHRR), Universal Mouse Reference RNA (UMRR) and Universal Rat Reference RNA (URRR), correspondingly. Fluorescent cDNA was synthesized by reverse transcription using allyl-amine modified dUTP, labeled with Cy5 or Cy3 and hybridized to 12,000 and 43,000-spot human, 7,500 and 8,000-spot mouse or 14,000-spot rat microarrays. Microarrays were scanned using an Axon scanner and analyzed using GenePix 3.0 and GeneTraffic (Iobion). RESULTS AND CONCLUSIONS. All three Universal Reference RNA demonstrated 80-99% hybridization coverage of different microarrays. Comparison of two lots each of UHRR, UMRR and URRR shows low variability with the correlation coefficients of 0.96. Evaluation of uniquely expressed genes in individual cell lines showed that each cell line contributes to the reference pool different sets of tissue-specific genes. The results of this study demonstrate the usefulness of Universal Reference RNA as a standard in two-color microarray experiment.

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Analysis for Differential mRNA Expression in U937 Cells after Exposure to Nicotine Using cDNA Microarray

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RATIONALE Cigarette smoking has been considered to play a significant role in a variety of respiratory disorders, especially in chronic obstructive lung disease or lung carcinomas. However, little is known how profoundly nicotine, a major pharmacological substance in cigarette smoke, would affect progression of these diseases. This study was undertaken to investigate direct influence on gene expressions in U937 cells, a human mononuclear-phagocyte cell line, after exposure to nicotine utilizing cDNA microarray analysis. *METHODS* We isolated total RNA from U937 cells which were stimulated by different concentrations of nicotine (1 μM ~ 1 mM), and converted to cDNA using random primers and ^{33}P-dATP. Then the plastic microarray carrying 12,000 different-length oligonucleotides were hybridized with the above ^{33}P-labeled cDNA probes. Positive signals were detected and analyzed by a standard phosphorimager. *RESULTS AND CONCLUSIONS* Expression of multiple genes such as those coding for growth factors or matrix metalloproteinases were up- or down- regulated by nicotine. These results suggest that exposure to nicotine could affect the lung cells directly through modulating the pattern of gene expressios, thereby would modify manifestations of a variety of respiratory diseases.

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