



Temporal Gene Expression Profiling in Compensatory Lung Growth

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Background

Pneumonectomy (removal of a lung) results in rapid, hyperplastic, compensatory growth of the remaining lung. The molecular mechanisms that regulate this regenerative growth are not well known. An understanding of these mechanisms and the role of different signaling pathways in this process could lead to therapies for lung injury, pulmonary hypertension, respiratory failure, transplantation for endstage lung disease, and even stimulation of regenerative growth in patients with minimal pulmonary tissues left after lung resection.

The goal of our study is to describe the signaling pathways that direct the compensatory lung growth after pneumonectomy.

Materials and Methods

Surgery: Mice (C57BL6 adult male, 10-12 weeks old) were anesthetized with inhaled halothane followed by endotracheal intubation and ventilation with room air. Left pneumonectomy was performed through a small left thoracotomy. The left hilum was isolated and ligated with a titanium clip and the left lung excised. Sham surgery consisted of left thoracotomy only. The chest was closed in layers and animals recovered under normal conditions.

RNA Extraction: At designated days after surgery, mice were anesthetized and harvested lungs (n=3/group for Gene Array and n=5/group for QRT-PCR) were stored in RNAlater at 4°C. Total lung RNA was isolated and the quality of the samples was assessed using the Agilent BioAnalyzer.

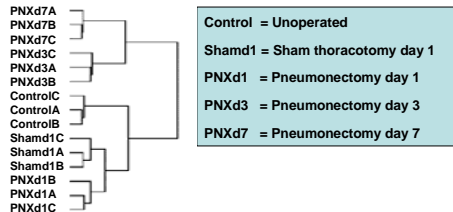
Gene Microarray: Gene array analysis was performed using Affimetrix MOE430_2 chips containing 45,037 gene probes. Cluster analysis and sample comparisons were carried out using the dChip software.

QRT-PCR: cDNA was generated by reverse transcription using ISCRIP. Specific primers were created using Beacon5 software. Real-time PCR was performed using 25ng cDNA in conjunction with SYBR Green Supermix.

Results

dChip Hierarchical Clustering

Hierarchical clustering is used to determine how closely the expression pattern of one sample matches the expression pattern of other samples. An unassisted hierarchical clustering created using dChip is shown below. Most of the samples cluster well with their replicates.



Results

dChip Comparisons

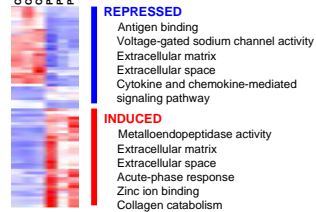
Two-group comparisons were also carried out using dChip software. The table below shows a summary of the comparisons performed and the gene expression differences observed compared to control.

	Upregulated					Downregulated						
	>10	5-10	3-5	2-3	1.5-2	Total	>10	5-10	3-5	2-3	1.5-2	Total
SHAMd1	0	7	16	21	10	54	0	1	1	14	8	24
PNXd1	1	7	18	10	7	43	1	1	6	17	8	33
PNXd3	3	5	24	36	15	83	1	6	52	103	23	185
PNXd7	1	37	62	87	39	226	1	0	8	16	26	51

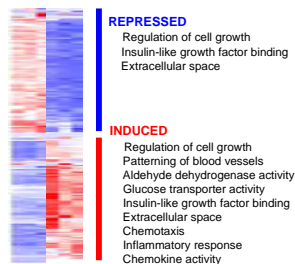
Pathway and Functional Analyses

The differentially expressed genes were examined for underlying pathways that may be responsible for the changes, as well as the high level functions involved in these changes. The genes identified as significantly changed were analyzed using the software *Ingenuity Pathway System*. The following "Heat Maps" illustrate those analyses in PNXd1, PNXd3 and PNXd7 groups where red color indicates upregulation and blue color indicates downregulation of gene expression.

PNXd1 Gene Ontology Categories

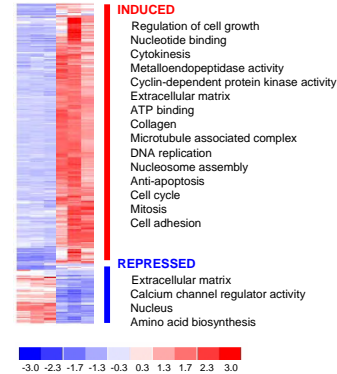


PNXd3 Gene Ontology Categories



Results

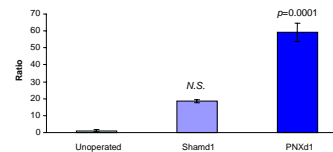
PNXd7 Gene Ontology Categories



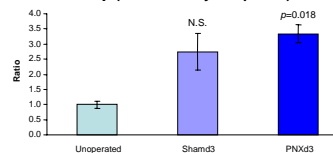
Validation – Real-Time PCR

Several specific genes were chosen to further validate using real-time PCR. Total RNA was reverse transcribed and amplified by PCR using primers to amplify specific sequences for 40 cycles using BioRad SYBR green Taq DNA polymerase. Amplification of the mouse beta 2 microglobulin was used as an internal control. Lungs from 0, 1, 3 and 7 days post-pneumonectomy were evaluated.

Elastin (Extracellular Matrix)

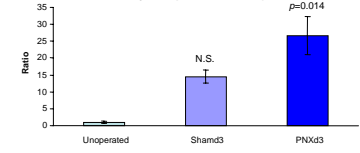


IL-1β (Inflammatory Response)

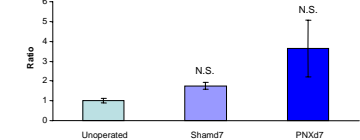


Results

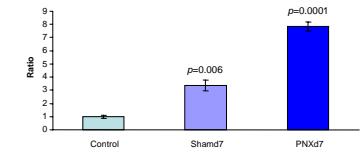
Cyr61 (Chemotaxis)



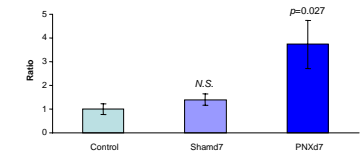
Cyclin A2 (Regulation of Cell Cycle)



IL-1β (Cell Proliferation)



MMP-12 (Metalloendopeptidase Activity)



Discussion

- This study highlights the complex signaling pathways during compensatory lung growth.
- The initial phase of the growth process is likely associated with inflammation and extracellular matrix catabolism.
- This is followed by signals for chemotaxis and cell growth.
- These signals (gene expressions) peak at day 7 with an enhanced proliferative and remodeling cellular response.