CHANGES IN RNA SUBCLASS EXPRESSION DURING LUNG DEVELOPMENT IN THE NITROFEN RAT MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA

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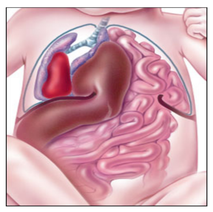
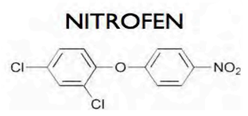
Background

Babies with congenital diaphragmatic hernia (CDH) have a hole in the diaphragm and abnormal lung development resulting in pulmonary hypoplasia and persistent pulmonary hypertension. CDH constitutes about 8% of major congenital anomalies.

There is no specific gene mutation associated with CDH. Therefore, we explore mechanisms of CDH pathogenesis using an epigenetic approach combined with miRNA-based strategies in the rat nitrofen model.





2,4-diclorophenyl-p-nitrophenyl ether

Hypothesis

Transcriptome changes contribute to abnormal lung development in the nitrofen rat model of CDH.

Objectives

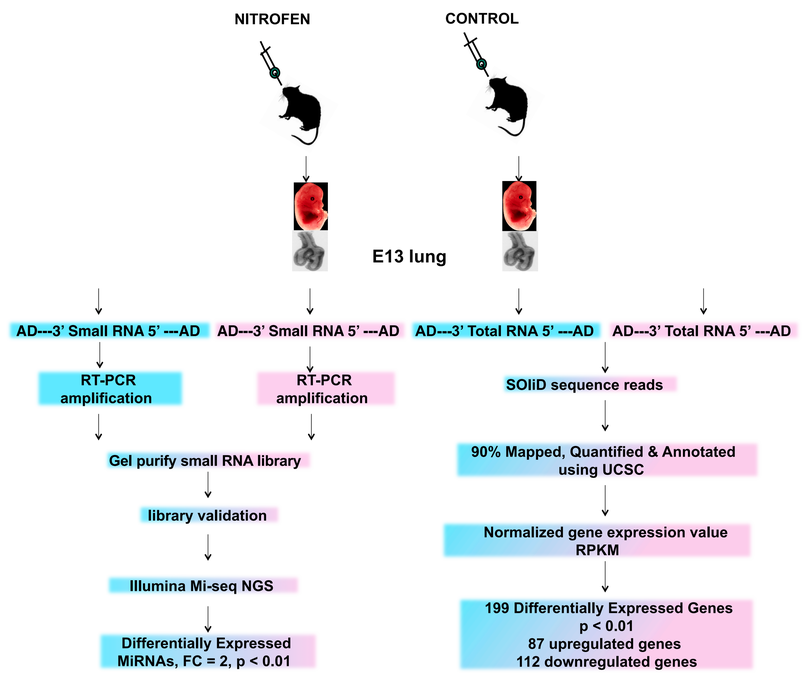
1. To profile significant changes in expression of RNA subclasses in embryonic nitrofen-induced abnormal lungs in rats
2. To analyze the similarity in structure and function of mRNA and miRNA reads between i) human and other species and ii) members of the same miRNA family
3. To identify the regulatory miRNA networks associated with nitrofen-induced abnormal lung development in the rat model of CDH.

Approaches

1. Correlating the differential changes in miRNA and mRNA expression
2. Phylogenetic inference analysis
3. Identifying human orthologous by similarity search
4. Identifying miRNA/mRNA biological (GO) terms, miRNA crosstalk with upstream or downstream regulatory factors. Notes : focus on the biology of nitrofen how it works?

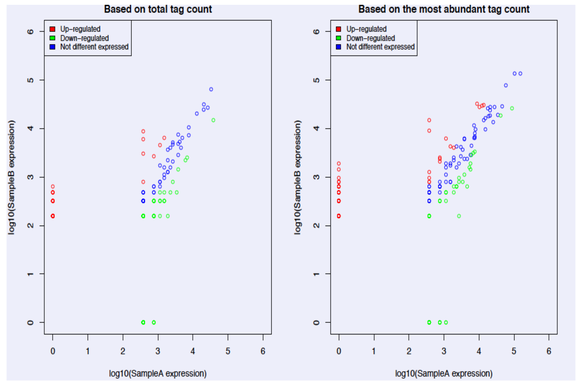
Methods and results

RNAseq-NGS workflow



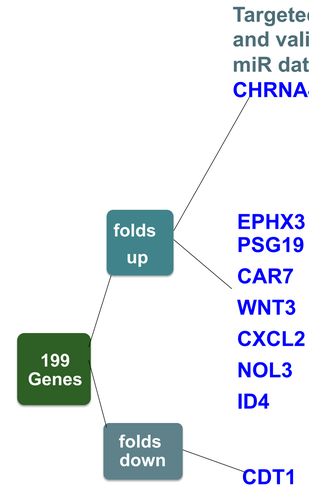
Nitrofen treatment (100 mg/ml olive oil) induced 87 mRNA genes by > 2 folds while 112 other genes were downregulated by at least 2 folds

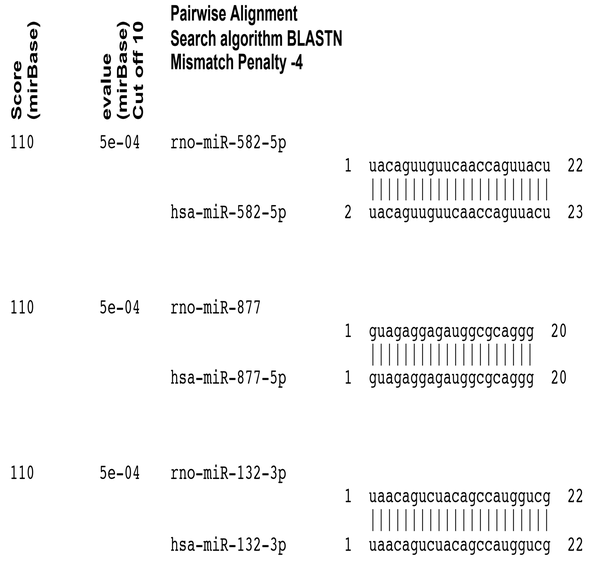
snRNA



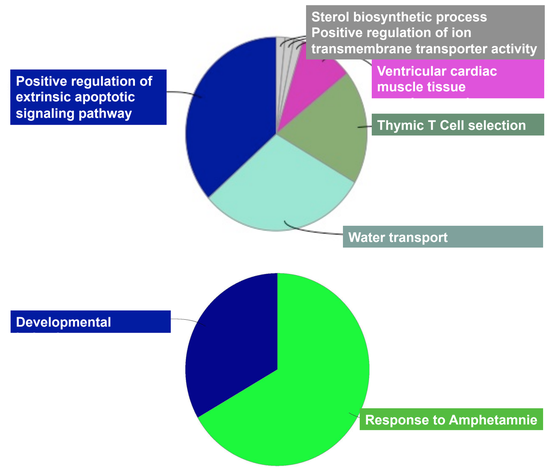
Differential expression between nitrofen and control. X-axis represents normalized control gene expression levels. The Y-axis represents normalized nitrofen gene expression levels. Each point in the scatter represents an individual snRNA. The points on both sides on the diagonal line represent a ratio of the normalized gene expression of the sample/the normalized gene expression of the control (the change in gene expression).

Validation of RNAseq by RT-qPCR





Rat miRNAs could function similarly to their orthologous in human and could help understand the effect of nitrofen not only on the mature structure of miRNA, but also on the entire miRNA-coding gene.



Example of a subset of gene reads after normalization and annotation

Conclusion

We observed the highest fold change in snRNA expression in response to nitrofen.

The nitrofen model helped identify three important biological functions of miRNAs throughout development:

1. Developmental inductions
2. Ventricular cardiac muscle tissue morphogenesis
3. Positive regulation of extrinsic apoptotic signaling

Significance

The obtained transcriptomes will help identify regulatory miRNA interactions with mRNA and/or other organogenesis networks associated with nitrofen-induced abnormal lung development in rats.

Acknowledgements

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