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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 leads all birth defects with the highest death rate among neonates. Nitrofen (2,4-diclorophenyl-p-nitrophenyl ether) is a pesticide that induces CDH and pulmonary hypoplasia in rodents in a strikingly similar pattern to humans. To date, the mechanism underlying nitrofen-induced lung hypoplasia is not well defined. We aimed to identify regulatory microRNA interactions with organogenesis networks associated with nitrofen in a rodent model of CDH.

Methods: We used Next Generation Sequencing technology to profile changes in expression of RNA subclasses from fetal rat lungs at embryonic day E13.5 upon treatment of dams with nitrofen or vehicle olive oil at E9. A dataset of significantly different mRNA, miRNA, tRNA, rRNA, snRNA and snoRNA were generated. After normalization, mRNA-encoding genes and miRNAs found to be significantly different were selected and identified through fold-change filtering. Selected genes were annotated for biological functions before a subsample was validated using RT-qPCR. Selected miRNAs and their corresponding human orthologous were identified using mirBase and mirOrtho databases. Rat miRNAs and their corresponding human orthologous sequences were clustered and analyzed for their structure conservation and function relatedness using a phylogeny inference approach.

Results: Nitrofen treatment induced 87 mRNA genes by more than 2 fold while 112 other genes were downregulated by at least 2 fold. mRNA gene expression was found to be organized into several clusters including extrinsic apoptotic signaling, thymic T cell selection, ventricular cardiac muscle tissue morphogenesis, water transport, sterol biosynthetic processes, iron transmembrane transporters, potassium ion transmembrane transport and was also involved in amphetamine treatment.

Conclusion: The obtained transcriptomes for control and nitrofen-induced hypoplastic lungs will help guide our future experiments to describe CDH-relevant miRNA evolutionary conservation between rats and humans and the expression correlation with their mRNA targets.