Seminars in Perinatology ▮ (2018) ▮▮▮–▮▮▮

Available online at www.sciencedirect.com

Seminars in Perinatology

www.seminperinat.com

Transcriptional profiling of the ductus arteriosus: Comparison of rodent microarrays and human RNA sequencing

Michael T. Yarboro[a], Matthew D. Durbin, MD[b], Jennifer L. Herington, PhD[c,d], Elaine L. Shelton, PhD[c,d], Tao Zhang, MD, PhD[e], Cris G. Ebby[f], Jason Z. Stoller, MD[e], Ronald I. Clyman, MD[g], and Jeff Reese, MD[a,c,]*

[a]*Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Vanderbilt University, 1125 Light Hall/MRB IV Bldg., 2215 B Garland Ave., Nashville, TN 37232*
[b]*Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, 46202*
[c]*Division of Neonatology, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232*
[d]*Department of Pharmacology, Vanderbilt University, Nashville, TN 37232*
[e]*Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, PA 19104*
[f]*Rutgers New Jersey Medical School, Newark, NJ 08901*
[g]*Department of Pediatrics, Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA 94143*

ARTICLE INFO

Keywords:
Ductus arteriosus
PDA
Microarray
RNA-seq
Transcriptional profiling
Aorta

ABSTRACT

DA closure is crucial for the transition from fetal to neonatal life. This closure is supported by changes to the DA's signaling and structural properties that distinguish it from neighboring vessels. Examining transcriptional differences between these vessels is key to identifying genes or pathways responsible for DA closure. Several microarray studies have explored the DA transcriptome in animal models but varied experimental designs have led to conflicting results. Thorough transcriptomic analysis of the human DA has yet to be performed. A clear picture of the DA transcriptome is key to guiding future research endeavors, both to allow more targeted treatments in the clinical setting, and to understand the basic biology of DA function. In this review, we use a cross-species cross-platform analysis to consider all available published rodent microarray data and novel human RNAseq data in order to provide high priority candidate genes for consideration in future DA studies.

Abbreviations: Ao, aorta; DEG, differentially expressed gene; bioDBnet, biological Database network; BP, biological process; CC, cellular component; DAVID, database for annotation, visualization, and integrated discovery; ECM, extracellular matrix; FDR, false discovery rate; FPKM, fragments per kilobase of transcript per million mapped reads; GO, gene ontology; GEO, gene expression omnibus; hg38, human genome version 38; RMA, Robust Multi-Array; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function; UP, UniProt; RIN, RNA Integrity Number

*Corresponding author at: Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Vanderbilt University, 1125 Light Hall/MRB IV Bldg., 2215 B Garland Ave., Nashville, TN 37232-0656. Tel.: +1 615 322 3476; fax: +1 615 343 6182.
E-mail address: jeff.reese@vanderbilt.edu (J. Reese).

https://doi.org/10.1053/j.semperi.2018.05.003
0146-0005/

Introduction

The ductus arteriosus (DA) is an essential vascular shunt connecting the pulmonary artery and aorta, allowing oxygenated blood from the placenta to bypass the developing lungs *in utero*. After birth, DA closure is required for a proper transition to neonatal life. Often, the postnatal DA fails to close, resulting in persistent patency of the ductus arteriosus (PDA). PDA accounts for nearly 10% of congenital heart defects,[1,2] including more than 30% of preterm infants with a birth weight of <1500 g.[3,4]

Effective DA closure is dependent on a combination of signaling and structural changes which support constriction and eventual remodeling of the vessel.[5–7] Despite the proximity and common neural crest lineage of their smooth muscle cells,[8] the ascending aorta (Ao) does not undergo these changes, suggesting transcriptional differences between these vessels may define the DA's function. Numerous attempts to understand these differences at the transcriptome level have provided insight, but varied experimental design and statistical analyses have created contradictions and ambiguity in the literature. Further, the transcriptome of the human DA has not been explored with the advanced genomic techniques now available, such as RNA-seq. A clear picture of the DA's transcriptional profile is key to guiding future research endeavors, both to allow more targeted treatments in the clinical setting, and to understand the basic biology underlying DA function.

The goals of this study were to (1) define differentially expressed genes (DEGs) in DA versus Ao samples that were commonly identified in previously published microarray datasets using rodent models, (2) identify human DA-enriched transcripts using RNA-seq analysis, and (3) explore transcriptional commonalities between the rodent and human DA. Although cross-species and cross-platform comparisons are fraught with limitations, identification of robust markers of DA identity or novel DA-enriched pathways promises to provide unique insights into DA development and function.

Methods

Microarray meta-analysis

Microarray data were obtained from NCBI's Gene Expression Omnibus (GEO) database (Table 1). All available studies were considered, but only investigations that included a DA/Ao comparison were selected for analysis. For the array data from Bokenkamp et al.,[9] values for E21 laser micro-dissected endothelium and smooth muscle cells were pooled. For the array data from Hsieh et al.,[10] only the F344 control samples were considered. CEL files of selected data sets were evaluated in Partek Genomics Suite version 7.17.1222 (Partek Inc.). All data were normalized using the Robust Multi-Array (RMA) method. One-way ANOVA was used to analyze contrasts of interest, namely vessel type, to generate lists of DEGs between DA and Ao. Permissive DEG lists (fold change $\geq$1.2) were then separated by increased or decreased DA/Ao expression to generate UP and DOWN lists, respectively. A naïve vote counting strategy was used to evaluate consistency between studies. Shared genes between UP lists or DOWN lists from each study were determined using Partek Genomics Suite.

RNA-seq analysis

Previable (21–21 5/7 weeks gestation) tissue samples for human RNA-seq analysis were obtained as previously described.[11] Tissues were homogenized in Trizol with the IKA T10 basic Ultra-Turrax. Total RNA was isolated using the RNeasy Mini kit (Qiagen). RNA quality was assessed using the Agilent 2100 Bioanalyzer. RNA samples with RNA Integrity Number (RIN) score $\geq$6.5 were considered for further study. 1 microgram of total RNA was used for library construction using the ScriptSeq Complete Gold Kit (Illumina). The libraries, four biological replicates per vessel type, were sequenced on an Illumina HiSeq 2000 by 100 bp pair-end sequencing. RNA-seq data was uploaded to Partek Flow (Partek Inc.). Trimming of raw reads (both ends) was based on a minimum read length of 25 and discarded bases after 85. Trimmed reads were aligned to Human Genome Version 38 (hg38) using STAR – 2.5.3a and quantified to Refseq Transcripts 83 using Partek's E/M method. Aligned counts were FPKM normalized with an offset of 1. Gene specific analysis (GSA) was used to detect DA vs. Ao DEGs. DEGs with an FDR of $\leq$0.1 and a fold change $\geq$2 were considered significant. Volcano plot and dendogram heat map figures were generated using Partek Flow.

Comparison of microarray and RNA-seq DEGs

Rodent gene symbols were converted to human orthologues using biological Database network (bioDBnet) to identify genes common between microarray and RNA-seq DEG lists. Lists were manually aligned to determine genes differentially expressed in both microarray and RNA-seq analyses. Microarray and RNA-seq gene lists were submitted independently for functional annotation using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Functional terms from Gene Ontology (GO) Biological Process (BP), GO Cellular Component (CC), GO Molecular Function (MF), Kyoto Encyclopedia of Genes and Genomes (KEGG), and UniProt (UP) Keywords databases were evaluated for similarities. Lists of functional terms and keywords from each database were then manually aligned. In order to create comparison diagrams, significant terms ($p \leq 0.05$) were chosen based on count (defined as number of genes identified in each term), and ordered by –Log (p value).

Results

Comparison of published rodent microarrays

Our microarray meta-analysis focused on studies containing DA to Ao comparisons in term animals, since there were too few preterm studies for comparison. Comparison of DA to Ao expression allowed DA-specific genes to be distinguished from temporally-regulated genes that are important for