Placenta 41 (2016) 74–82

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Comparing human and macaque placental transcriptomes to disentangle preterm birth pathology from gestational age effects

Haley R. Eidem [a], David C. Rinker [a, b], William E. Ackerman IV [c], Irina A. Buhimschi [d], Catalin S. Buhimschi [c], Caitlin Dunn-Fletcher [e], Suhas G. Kallapur [d], Mihaela Pavličev [e], Louis J. Muglia [e], Patrick Abbot [a], Antonis Rokas [a, b, *]

[a] Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA
[b] Program in Human Genetics, Vanderbilt University, Nashville, TN 37235, USA
[c] Department of Obstetrics and Gynecology, The Ohio State University, Columbus, OH 43210, USA
[d] Center for Perinatal Research, Nationwide Children's Hospital, Columbus, OH 43210, USA
[e] Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH 45229, USA

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form
5 March 2016
Accepted 10 March 2016

Keywords:
Pregnancy
Placenta
Preterm birth
Gestational age
RNA sequencing
Transcriptomics

ABSTRACT

Introduction: A major issue in the transcriptomic study of spontaneous preterm birth (sPTB) in humans is the inability to collect healthy control tissue at the same gestational age (GA) to compare with pathologic preterm tissue. Thus, gene expression differences identified after the standard comparison of sPTB and term tissues necessarily reflect differences in both sPTB pathology *and* GA. One potential solution is to use GA-matched controls from a closely related species to tease apart genes that are dysregulated during sPTB from genes that are expressed differently as a result of GA effects.
Methods: To disentangle genes whose expression levels are associated with sPTB pathology from those linked to GA, we compared RNA sequencing data from human preterm placentas, human term placentas, and rhesus macaque placentas at 80% completed gestation (serving as healthy non-human primate GA-matched controls). We first compared sPTB and term human placental transcriptomes to identify significantly differentially expressed genes. We then overlaid the results of the comparison between human sPTB and macaque placental transcriptomes to identify sPTB-specific candidates. Finally, we overlaid the results of the comparison between human term and macaque placental transcriptomes to identify GA-specific candidates.
Results: Examination of relative expression for all human genes with macaque orthologs identified 267 candidate genes that were significantly differentially expressed between preterm and term human placentas. 29 genes were identified as sPTB-specific candidates and 37 as GA-specific candidates. Altogether, the 267 differentially expressed genes were significantly enriched for a variety of developmental, metabolic, reproductive, immune, and inflammatory functions. Although there were no notable differences between the functions of the 29 sPTB-specific and 37 GA-specific candidate genes, many of these candidates have been previously shown to be dysregulated in diverse pregnancy-associated pathologies.
Discussion: By comparing human sPTB and term transcriptomes with GA-matched control transcriptomes from a closely related species, this study disentangled the confounding effects of sPTB pathology and GA, leading to the identification of 29 promising sPTB-specific candidate genes and 37 genes potentially related to GA effects. The apparent similarity in functions of the sPTB and GA candidates may suggest that the effects of sPTB and GA do not correspond to biologically distinct processes. Alternatively,

Abbreviations: PTB, preterm birth; sPTB, spontaneous, idiopathic preterm birth; GA, gestational age; FPKM, fragments per kilobase per million base pairs.

* Corresponding author. Department of Biological Sciences, Vanderbilt University, VU Station B #35-1634, Nashville, TN 37235, USA.

E-mail addresses: haley.eidem@vanderbilt.edu (H.R. Eidem), david.rinker@vanderbilt.edu (D.C. Rinker), william.ackerman@osumc.edu (W.E. Ackerman), irina.buhimschi@nationwidechildrens.org (I.A. Buhimschi), catalin.buhimschi@osumc.edu (C.S. Buhimschi), caitlin.dunn@cchmc.org (C. Dunn-Fletcher), suhas.kallapur@cchmc.org (S.G. Kallapur), mihaela.pavlicev@cchmc.org (M. Pavličev), louis.muglia@cchmc.org (L.J. Muglia), patrick.abbot@vanderbilt.edu (P. Abbot), antonis.rokas@vanderbilt.edu (A. Rokas).

http://dx.doi.org/10.1016/j.placenta.2016.03.006

it may reflect the poor state of knowledge of the transcriptional landscape underlying placental development and disease.

1. Introduction

Preterm birth (PTB), or birth before 37 completed weeks of gestation in humans, is a global health issue affecting at least 15 million newborns every year [1–3]. This complex, multifactorial syndrome accounts for around 1 million neonatal deaths annually and surviving neonates often require lifelong care for common comorbidities including developmental, visual, and digestive problems [4,5]. 30% of PTB cases are indicated by medical conditions such as preeclampsia or intrauterine growth restriction, while the remaining 70% are caused by the spontaneous onset of labor either with (25%) or without (45%) premature membrane rupture [6,7].

Spontaneous, idiopathic preterm birth (sPTB), much like most other complex human genetic diseases, is augmented by environmental risk factors (e.g., stress, infection, and socioeconomic status) as well as by genetics. Several studies have shown that women are more likely to deliver preterm if a sister delivered preterm, if a previous child was born preterm, if they were born preterm themselves, or if they have African American ancestry [8–11]. In recent years, studies have also highlighted the importance of gene expression regulation in complex genetic diseases [12]. Thus, analysis of the genetic elements that are active or dysregulated in gestational tissues harbors great potential to identify candidate genes for sPTB and several genome-wide studies have already started to outline its genomic, transcriptomic, and methylomic architecture [13].

Nevertheless, a major obstacle in the transcriptomic study of sPTB in humans is the inability to collect gestational age-matched healthy control tissue to compare with pathologic preterm tissue. Without safe, non-invasive procedures to sample healthy preterm tissues destined for healthy term births, the most common approach is to use healthy term tissues as the control for pathologic preterm tissues [14–16]. This complicates downstream data analysis, though, because observed differences in gene expression reflect not only differences in pathology, but also differences in gestational age (GA).

One potential solution is to use GA-matched controls from a closely related species to distinguish genes dysregulated during sPTB from genes expressed differently at different points in pregnancy. The decoding of the rhesus macaque (*Macaca mulatta*) genome and subsequent comparison with that of human and chimpanzee revealed that these 3 primate species share about 93% of their DNA [17]. Thus, macaque is an ideal species for transcriptional comparison with humans not only because the two species share a close evolutionary affinity but also because of similarities with respect to key pregnancy-related traits. For example, even though placental morphology is highly variable across mammals, human and macaque placentas share the same discoid shape, hemochorial invasiveness, and villous interdigitation [18,19]. Similarly, the relationship between pelvis and fetal head size in humans is more akin to the relationship in macaques than it is to any other primates [20]. This is particularly important as it would alleviate any effects that cephalopelvic constraints might have on birth timing [21]. Finally, several human pregnancy pathologies have been recorded in macaques including stillbirth, PTB, placenta previa, and placental abruption [22].

In this study, we compare transcriptomes from human sPTB placentas, human term placentas, and macaque placentas at 80% completed gestation to distinguish between sPTB-specific and GA-specific candidate genes. Specifically, candidate genes that are differentially expressed between human sPTB and human term as well as between human sPTB and macaque are potentially sPTB-specific. In contrast, candidate genes that are differentially expressed between human sPTB and human term as well as between human term and macaque are potentially GA-specific. This novel comparative approach disentangles the confounding effects of sPTB and GA differences and allows for the educated prioritization of candidate genes for future studies of pregnancy and prematurity.

2. Methods

2.1. Tissue collection, RNA isolation, and RNA sequencing

Human placentas were collected immediately after delivery and the decidua basalis layer from a central cotyledon was dissected and discarded. Approximately 1 g of underlying villous tissue was then biopsied for further analysis. The 5 term (GA 38–39 weeks, mean 38 or 95% completed gestation) human placental tissue biopsies were all collected after cesarean delivery. Of the 5 preterm (GA 29–33 weeks, mean 32 or ~80% completed gestation) human placental tissue biopsies that were collected, 4 were collected after cesarean delivery and 1 after vaginal delivery. Each of the biopsies was flash frozen in liquid nitrogen and stored at −80 °C [23]. Total RNA was isolated using TRIzol and Illumina libraries were constructed using the TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero Gold. RNA sequencing (RNA-seq) was performed on an Illumina HiSeq 2500 machine using HiSeq version 3 sequencing reagents. The samples were sequenced using a single-end approach with 50 bp reads, generating approximately 30 million reads per sample. Raw count data have been deposited in the NCBI Gene Expression Omnibus (GEO) database and are accessible through GEO Series accession number GSE73714 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73714).

Macaque placentas were collected immediately after delivery and full thickness biopsies (~2cmx2cm) free of clots and debris were taken midway between the attachment of the umbilical cord and the placenta edge. Both placental tissue samples were collected after cesarean delivery via hysterotomy (GA 128–131 days, mean 129.5 or ~80% completed gestation) [24]. Total RNA was isolated from 100 μg of frozen tissue using TRIzol and suspensions were stored at −80 °C. RNA-seq was performed on an Illumina HiSeq machine after passing initial quality control metrics. The two samples were sequenced using a paired-end approach with 50 bp reads, generating approximately 15 million paired reads per sample.

2.2. Data processing and differential expression analysis

RNA-seq data was analyzed from the 5 term and 5 preterm human placental tissue samples as well as the 2 macaque placental samples from 80% completed gestation. Raw sequence read files were first quality checked using FastQC and then trimmed for