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A comprehensive analysis of the human placenta transcriptome

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ABSTRACT

As the conduit for nutrients and growth signals, the placenta is critical to establishing an environment sufficient for fetal growth and development. To better understand the mechanisms regulating placental development and gene expression, we characterized the transcriptome of term placenta from 20 healthy women with uncomplicated pregnancies using RNA-seq. To identify genes that were highly expressed and unique to the placenta we compared placental RNA-seq data to data from 7 other tissues (adipose, breast, hear, kidney, liver, lung, and smooth muscle) and identified several genes novel to placental biology (*QSOX1*, *DLG5*, and *SEMA7A*). Semi-quantitative RT-PCR confirmed the RNA-seq results and immunohistochemistry indicated these proteins were highly expressed in the placental syncytium. Additionally, we mined our RNA-seq data to map the relative expression of key developmental gene families (Fox, Sox, Gata, Tead, and Wnt) within the placenta. We identified *FOXO4*, *GATA3*, and *WNT7A* to be amongst the highest expressed members of these families. Overall, these findings provide a new reference for understanding of placental transcriptome and can aid in the identification of novel pathways regulating placenta physiology that may be dysregulated in placental disease.

1. Introduction

The placenta is a specialized temporary fetal organ that is requisite for fetal growth and development in eutherian mammals and is crucial for a successful pregnancy. Early in pregnancy the placenta is responsible for implantation, anchoring of the embryo into the uterine wall, and producing hormones that initiate maternal recognition of pregnancy. Throughout gestation, placental development proceeds via an elegantly orchestrated regulation of trophoblast invasion, proliferation and differentiation, as well as, vasculogenesis and angiogenesis to ensure adequate blood supply to support placental and fetal growth. Thereafter, the placenta provides the sole conduit for transferring maternally derived nutrients and gasses to the ever-demanding fetus.

Disruptions to placental development in the first half of pregnancy and/or insufficient adaptive changes in the placenta in response to maternal environment during the latter half of pregnancy have been associated with a number of pregnancy complications and compromised fetal outcomes. In this context, defining placenta specific gene expression can contribute to the understanding of placenta development and function. Unlike microarrays, which have been the primary tool to-date to study placental gene expression [1–4], RNA-seq allows digital quantitation of gene expression data and hence allows assessment of relative abundance between genes within and between samples. Here, we set out to identify genes that are specific to human placenta using deep sequencing technology in order to increase general understanding of placental function and development.

2. Methods

Collection of term placental samples: Placenta tissue was collected at term at the University of Arkansas for Medical Sciences following informed consent from mothers. The protocol was approved by the Institutional Review Board at UAMS (NCT01104454). Included in this study were non-smoking mothers without gestational diabetes, pre-eclampsia or other complications who had either vaginal or cesarean deliveries (Table 1).

Preparation, sequencing, and data analysis of RNA-seq libraries: Total RNA was isolated from 20 placenta (pooled from 6 separate locations) using RNeasy columns [5] including DNase digestion. cDNA libraries were prepared using NebNext reagents as previously described [5]. Single-read 36-bp sequencing was performed using a GAII$_X$ (Illumina). RNA-seq data for other tissues (liver, skeletal muscle, adipose, kidney, lung breast, and heart) were acquired in FASTQ format from the Illumina BodyMap 2.0 project. Alignment to the human genome (hg19) was carried out using Bowtie [6]. All data were analyzed in Avadis-NGS and SeqMonk software packages.

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Table 1
Maternal characteristics.

	Mean ± SE	[Min, max]
n	20	–
Age (yrs.)	29.2 ± 1.4	[18, 43]
Weight (lb.)	179.7 ± 11.5	[110, 277]
Height (in.)	65.6 ± 0.7	[60, 72]
BMI (kg/m^2)	29.3 ± 1.8	[18.3, 43.4]
Parity (n)	2.1 ± 0.3	[0, 5]
Placenta weight (g)	659 ± 29.1	[459, 951]
C-Section (n)	11	–
Fetal sex	7-F, 13-M	–

n = number, F = female, M = male.

Uniquely aligned reads were quantified in Avadis-NGS and gene-level reads per kilobase per million mapped reads (RPKM) values were calculated. Pair-wise comparisons between placenta and individual tissues were carried out ($p < 0.05$, Audic-claverie test; and 3-fold higher expression in placenta). Corrections for multiple testing were performed using the false discovery rate method [7]. The intersection of these comparisons was used for hierarchical clustering using R-Bioconductor and for functional enrichment using TargetMine. Visualizations were performed using Circos [8].

Quantitative RT-PCR: Total RNA was isolated from placenta as described above while RNA for other tissues was obtained commercially from Clontech (#636643, #636576, and #636558, Mountain View, CA). Total RNA (1 μg) was reverse transcribed using IScript cDNA synthesis kit and subsequent real-time PCR analysis was performed using an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA). Gene specific primers were designed using Primer Express Software (Applied Biosystems) for *QSOX1* (forward: 5′-GACTGTGCTGAGGAGACCAACA-3′, reverse: 5′-CCCGAGCCGTTCTTGGTAA-3′), *DLG5* (forward: 5′-AAGGCGGAACGCATTAAAATC-3′, reverse: 5′-TGCATTCAGAATGTGACACTGAAC-4′) and *SEMA7A* (forward: 5′-TCATGTCCCGAGACCCCTACT-3′, reverse: 5′-GTGTGGCTCGGCTGGATTAAT-3′). The relative amounts of mRNA were quantified using the ΔCT method and normalized to the expression of *cyclophilin A* mRNA [5].

Immunohistochemistry: Placenta tissues were fixed over-night in 3% paraformaldehyde, dehydrated through 5–15% sucrose, and frozen in OCT. Five micron sections were air dried and subjected to antigen retrieval (#45080-9K, Biogenex, San Ramon, CA). Immunolocalization was carried out using VectaStain Elite reagents (Vector labs). Briefly, sections were blocked with either rabbit or goat serum, followed by incubation with primary antibodies: QSOX1 #HPA042127 (Sigma, St. Louis, MO); DLG5 #ab56492 (Abcam, Cambridge, MA); SEMA7A #sc-374432 (Santa Cruz Biotechnology, Dallas, TX); FoxO4 #9472 (Cell Signaling, Danvers, MA); GATA-3 #sc-9009 (Santa Cruz Bio.); Wnt-7a #HPA015719 (Sigma). Primary antibodies were used at 1:100 concentrations. Controls excluding primary antibodies were used for all staining procedures. Incubations were carried out for 2 h at room temperature. Visualization of staining was performed using diaminobenzadine (Dako).

3. Results and discussion

This dataset included ~200 million reads covering 20 biological replicates. Quantitation of reads over genes revealed that ~80% of all annotated UCSC genes had at least one read and ~54% showed RPKM values > 1. We ranked genes based on transcript abundance in the placenta (top 100 shown in track 2 of Fig. 1A). High-resolution images are provided in Supplementary material. Consistent with a previous study where microarray analysis was used to find placental specific genes [2], many of the top expressed placental genes have been shown to regulate placental and fetal growth and have been identified as markers for placental diseases. For example *H19* and *IGF2*, both imprinted genes that produce potent growth factors [9], had a RPKM of >1000. *HTRA1* was also highly expressed with a RPKM of ~300. *HTRA1* encodes a peptidase that may play a role in the regulation of IGF bioavailability by cleaving IGF-binding proteins [10] and is dysregulated in trophoblastic diseases [11]. Additionally, GDF15 is a transforming growth factor-beta (TGF-β) cytokine that has implications in cardiovascular disease and has been identified to be dysregulated in pre-eclamptic and diabetic pregnancies [12]. *GDF15* also had a RPKM of >1000 and was within the top 20 expressed genes in the placenta.

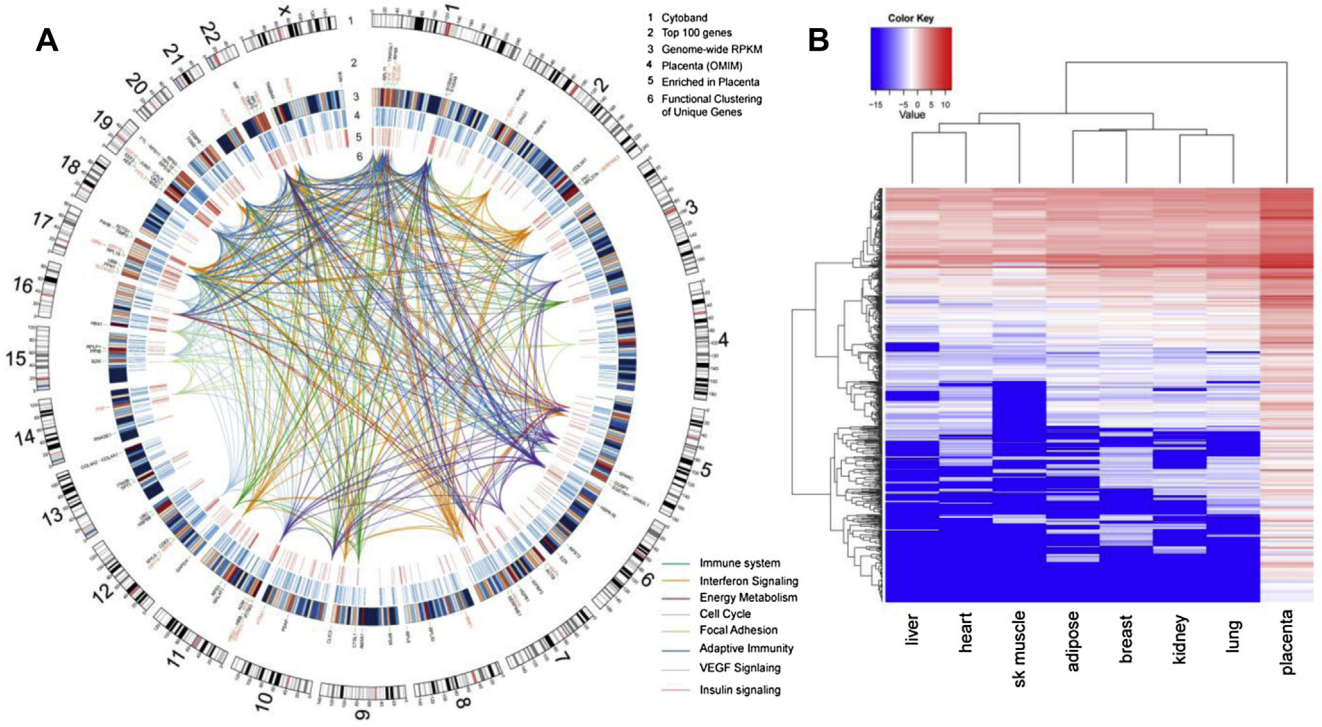

Fig. 1. RNA-seq of human term placenta ($n = 20$) **A**) Circos diagram depicting whole-genome RNA-seq data. *Track 1*: cytoband, chromosomes are depicted qter to pter. *Track 2*: Genomic location of top 100 highly expressed genes in placenta based on average RPKM values. Gene names in red represent genes specifically enriched in placenta. *Track 3*: Average RPKM values summarized over 6 MB regions showing regions of high gene expression. *Track 4*: Locations of genes related to placenta in the OMIM database. *Track 5*: Genes specifically enriched in placenta (3-fold over 7 other tissues); *Track 6*: Biological functions enriched among placenta-enriched genes. High-resolution images are provided in supplementary material. **B**) Hierarchical clustering of the 288 genes that were at least 3-fold higher and had an RPKM >1 in the placenta compared to liver, heart, smooth muscle, adipose, breast, kidney, and lung. High expression is represented in red and low expression in blue.