Placental transcriptome in development and pathology: expression, function, and methods of analysis

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The placenta is the essential organ of mammalian pregnancy and errors in its development and function are associated with a wide range of human pathologies of pregnancy. Genome sequencing has led to methods for investigation of the transcriptome (all expressed RNA species) using microarrays and next-generation sequencing, and implementation of these techniques has identified many novel species of RNA including: micro-RNA, long noncoding RNA, and circular RNA. These species can physically interact with both each other and regulatory proteins to modify gene expression and messenger RNA to protein translation. Transcriptome analysis is actively used to investigate placental development and dysfunction in pathologies ranging from preeclampsia and fetal growth restriction to preterm labor. Genome-wide gene expression analysis is also being applied to identify prognostic and diagnostic biomarkers of these disorders. In this comprehensive review we summarize transcriptome biology, methods of isolation and analysis, application to placental development and pathology, and use in diagnostic analysis in maternal blood. Key information for analysis methods is organized into quick reference tables where current analysis techniques and tools are cited and compared. We have created this review as a practical guide and starting reference for those interested in beginning an investigation into the transcriptome of the placenta.

Key words: microarray, next-generation sequencing, placenta, RNA, transcriptome

The transcriptome is defined as the entire collection of RNA transcripts produced from the genome of a cell in any given state. Over the last decade, the advent of RNA-sequencing (RNA-seq) technologies, coupled with computational analysis, has greatly expanded our knowledge of the importance of the larger transcriptome.[1] Until recently, the central dogma in cell biology considered gene expression to be a linear 3-step process, whereby DNA is transcribed into messenger RNA (mRNA), which in turn is subsequently translated into a specific protein. In this paradigm, mRNA is viewed as a passive step in protein formation. Acknowledging the broader transcriptome provides a much more active and dominant role for the family of RNAs in regulating cell biology during normal development and specific pathologies of the placenta.[2-4] RNA species are extraordinarily diverse in structure, size, and function, with the discovery of regulatory noncoding RNAs (microRNAs [miRNAs], long noncoding RNAs [lncRNAs], and circular RNAs [circRNAs]),[2-4] and differences in mRNA exon splicing and 5' and 3' untranslated regions (UTRs),[5,6] highlighting the importance and complexity of gene expression (Figure 1). Gene regulation in development is a complex process and assessment of any 1 biomolecule can only tell part of the story.[7] Similarly the heterogeneous diseases of pregnancy are multifactorial and require large-scale unbiased approaches to gene expression analysis to identify different molecular classes of these pathologies.[8,9] Therefore, a comprehensive understanding of the changes that occur in the transcriptome during development and pathology of the placenta should be considered essential in identifying the origins of the placental basis of diseases that cause stillbirth or iatrogenic preterm birth. This is coupled with the profound influences adverse pregnancy can have on developmental programing for postnatal life and classification of future risk of chronic disease.[10-12]

In this review, we discuss mRNA, miRNA, lncRNA, and circRNA; their regulatory effects on gene expression; and methods for their isolation and analysis. Furthermore, placenta pathologies compared to other complex pathologies such as breast cancer are understudied. Specific to the human placenta there are 132 published data series, which is 10 times fewer than the 1402 series available for breast cancer. For this reason, the review is organized by RNA molecule to give placenta-specific examples as not all pathologies of pregnancy involving the placenta have been assessed for all classes of RNA molecules.

Our review focuses on human examples, as these are the most clinically relevant data sets. However, we would like to acknowledge the large amount of work performed on the placenta transcriptome of nonhuman species that has led to significant insights into placenta development, pathology, and evolution.[13-15]

MiRNAs

MiRNAs are a diverse and abundant class of short noncoding RNAs with spatial, temporal, and tissue-specific expression patterns.[3] MiRNAs can be self-contained genes transcribed by RNA polymerase II or III, or they can be located in the introns of protein coding genes, in which case they are released

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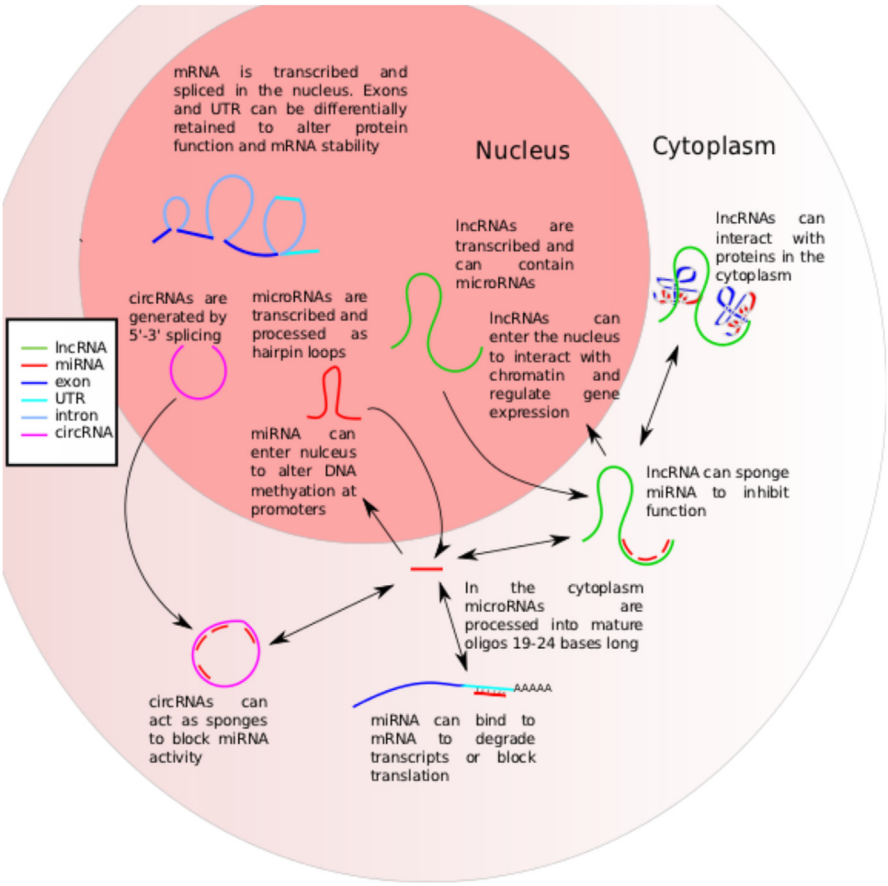
through the phenomenon of alternative splicing.[6] Their transcripts contain a hairpin loop essential for their processing and export from the nucleus to the cytoplasm.[16]

The first discovered regulatory role of miRNAs was as posttranscriptional repressors of their own mRNA targets by complementary binding of 3'-UTRs based on base pairing with a seed sequence.[17] This process is not entirely understood and most major databases computationally predict binding targets, which in some cases are supported by in vitro assays. Additionally, the small size of the seed sequence (6-8 bases) results in redundancy between different miRNAs. An exciting recent discovery is that miRNAs can also function as transcriptional inhibitors[18] through promoter targeting and chromatin remodeling.[19]

Significantly, multiple clusters of primate-specific miRNAs in the genome have been identified to have specific expression in the placenta and trophoblast.[20,21] This may be a fundamental requirement as rodents also have family-specific clusters of miRNAs uniquely expressed in the placenta trophoblast.[22] These could be involved in intrinsic cellular processes, but may also have a role in regulation of maternal adaptation to pregnancy through secretion via exosomes.[23,24] These exosomes are picked up by maternal cells (eg, endothelial cells) and can affect their gene expression environment.[25,26] Analysis of miRNA changes in expression in placenta pathologies should be studied in the context of both retained and secreted miRNAs.

FIGURE 1
Interactions and functions of cellular transcriptome



All RNA species are transcribed in the nucleus, but can be differentially processed and exported. MicroRNAs (miRNAs) and long noncoding RNAs (lncRNAs) can also be imported back into the nucleus where they can affect gene regulation through chromatin structure and epigenetic modifications. Complete functions of lncRNAs and circular RNAs (circRNAs) are still being elucidated.

mRNA, messenger RNA; *UTR*, untranslated region.

Cox. Placental transcriptome analysis. Am J Obstet Gynecol 2015.

LncRNA

LncRNAs have a length of >200 nucleotides, and display developmentally regulated tissue and cell type−specific expression.[2,27] LncRNAs can be transcribed as whole or partial antisense transcripts of coding genes. In the trophoblast of the placenta, the best-known example is H19, with conserved expression in mouse and human.[28] H19 contains a miRNA that may be processed and released.[29] and may regulate placental/fetal growth.[28,30] Generally, lncRNAs have been shown to bind DNA in a sequence-specific manner; a classic example of which is XIST that functions in X chromosome inactivation in females,[31] a process that is paternal specific in the trophoblast and random in the fetus.[32] They can also mark transcriptional activity,[33] and modulate protein activity and localization,[2] and epigenetic regulation.[34] LncRNAs can contain multiple seed sequences recognized by miRNAs and may therefore affect their function by acting as sponges or "sinks" to sequester miRNAs from their target mRNAs.[2] Recently, lncRNAs have also been shown to reenter the nucleus and associate with chromatin, and as such, may serve to anchor or guide epigenetic modifications of DNA through RNA-DNA base pairing.[34,35] Unfortunately, their exploration of expression and function in the placenta and trophoblast has been limited both in experimental scope and interpretation due to their diversity of proposed functions and poor annotation.

CircRNAs

Once thought to be an aberration of splicing pathology,[36] circRNAs are found in all phyla, are diverse (25-100 thousand unique members), and can far outnumber linear RNAs.[37,38] These