MicroRNAs in placental health and disease

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MicroRNAs (miRNAs) constitute a large family of small noncoding RNAs that are encoded by the genomes of most organisms. They regulate gene expression through post-transcriptional mechanisms to attenuate protein output in various genetic networks. The discovery of miRNAs has transformed our understanding of gene regulation and sparked intense efforts intended to harness their potential as diagnostic markers and therapeutic tools. Over the last decade, a flurry of studies has shed light on placental miRNAs but has also raised many questions regarding the scope of their biologic action. Moreover, the recognition that miRNAs of placental origin are released continually in the maternal circulation throughout pregnancy suggested that circulating miRNAs might serve as biomarkers for placental function during pregnancy. Although this generated much enthusiasm, recently recognized challenges have delayed the application of miRNA-based biomarkers and therapeutics in clinical practice. In this review, we summarize key findings in the field and discuss current knowledge related to miRNAs in the context of placental biology.

Key words: adverse pregnancy outcome, C19MC, exosomes, miRNA, placenta, pregnancy complications, primate-specific miRNA, trophoblast, trophoblast-specific miRNA

The recent finding of pervasive transcription across the genomes of all kingdoms of life challenges some long-held ideas regarding the genome and its regulation. A consequence of this widespread transcription is the production of numerous RNA transcripts with relatively unknown functions. A large fraction of these transcribed RNAs are not translated into proteins but exhibit regulatory functions that increasingly are recognized as critical factors in development and homeostasis. A major class of these noncoding RNAs, and one of the best studied, is the family of small regulatory RNAs called microRNAs (miRNAs). MiRNAs originally were described in the nematode *Caenorhabditis elegans* and were later found in the genomes of protists, plants, animals, and viruses, with the notable exception of bacteria. MiRNAs are single-strand RNA molecules of 20-24 nucleotides that usually repress gene expression by guiding an RNA-induced silencing complex (RISC) that contains argonaute (Ago) proteins (Table) to a target RNA, which they bind through imperfect base-pairing. Gene expression then is attenuated to a variable degree by the inhibition of the messenger RNA (mRNA) translation and transcript destabilization, which results in reduced protein synthesis. Interestingly, although most miRNAs exert a modest effect on individual targets,[1,2] perturbations in miRNA expression levels can have marked biologic consequences. Indeed, a growing list of miRNAs have been implicated in the pathogenesis of human diseases, which include but is not limited to cancer, cardiovascular disease, liver and kidney diseases, and psychiatric disorders.[3-7] Tissue expression of these miRNAs commonly is quantified with the use of polymerase chain reaction, northern blot, microarrays, and RNA sequencing.

To date, the biological database miRBase, which was developed by the Griffiths-Jones Laboratory at the Faculty of Life Sciences, University of Manchester,[8] contains more than 2500 entries for human miRNAs, although that number might be an overestimation because some of the species represent computer-based predictions without experimental validation.[9,10] Different cell types express common and unique miRNA species, and miRNA expression patterns are influenced by developmental and pathologic states. The human placenta expresses a distinct miRNA repertoire that is characterized by the fact that a large proportion of miRNAs are derived from the 2 largest clusters of miRNAs in humans, the chromosome 14 miRNA cluster (C14MC) and the chromosome 19 miRNA cluster (C19MC).[11] Although the functions of placental miRNAs are largely unknown, recent research has begun to shed light on their role in placental biology, as detailed later in the article. Likewise, the finding that placental miRNAs are released into the maternal circulation has raised the exciting prospect of the use of miRNA expression profiles as noninvasive markers of placental dysfunction. In this review, we briefly describe how miRNAs are produced and summarize recent developments in our understanding of the biological action of miRNAs in the human placenta.

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TABLE
Glossary of terms

Term	Explanation
Argonaute	A family of proteins characterized by a specific structural organization and a critical role in the silencing process by miRNAs. Argonaute 2 (Ago2), for example, is a part of the RNA-induced silencing complex (RISC) and is responsible for the cleavage of the target mRNA.
Canonical pathway	The prototypical pathway of a biologic process. Noncanonical refers to pathways that deviate from the canonical pathway or represent a less frequent or less known alternative.
Dicer	An RNA-specific enzyme that cleaves a pre-miRNA (and other types of double-stranded RNAs) into 21-24-nucleotide long double stranded RNAs with a 2-base overhang at the 3′ end.
Drosha	A nuclear RNA-specific enzyme that processes newly transcribed primary miRNA to produce a ~70 base pairs transcript with a hairpin shape, called pre-miRNA.
Endonucleolytic cleavage	The enzymatic cleavage of nucleic acid molecules through the hydrolysis of internal covalent bonds between nucleotides.
Exosomes	Small vesicles (50-150 nm) that are released into the extracellular environment when endosomal multivesicular bodies fuse with the plasma membrane.
Exportin 5	A nuclear envelope protein that mediates the nuclear export of pre-miRNAs to the cytoplasm; this process is assisted by the protein cofactor Ran-GTP (see later).
Microprocessor	A protein complex consisting of a catalytic core made of the Drosha nuclease and the RNA-binding protein DGCR8 (DiGeorge syndrome critical region 8).
Mirtrons	A subpopulation of miRNAs that are located in the introns of genes and produced by an alternative synthesis pathway, independent of the Drosha enzymatic complex.
Ran GTPase	A member of the family of GTPase enzymes that is involved in many nucleocytoplasmic transport pathways by regulating the interactions of protein carriers with their cargo.
RNA polymerase II (RNA pol II or RNAP II)	An enzyme that orchestrates the transcription of DNA into RNA or miRNA molecules.
RNase III	An RNA-specific endonuclease that cleaves double-stranded RNA molecules. Drosha and Dicer are members of this family.
Stem-loop	A secondary structure in DNA or RNA molecules that occurs when a strand folds and form a intramolecular base pairing with another section of the same strand, creating a U-shape structure.
Spliceosome	A large ribonucleoprotein complex involved in the removal of introns from unprocessed mRNAs in eukaryotic cells.
TRBP	A double strand RNA binding protein that is an essential interacting partner of Dicer in the biogenesis of miRNAs.

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The discovery of miRNAs

MiRNAs were discovered in the nematode *C elegans* by the groups of Lee et al[12] and Wightman et al[13] while they were studying a pair of developmental genes. One of these genes, lin-14, controls stage-specific cell lineages during larval development and was known to be itself regulated by the lin-4 gene. Lin-4 does not encode a protein product but instead gives rise to a small RNA transcript of 22 nucleotides with complementarity to 3′-untranslated regions of the lin-14 mRNA.[12] These 3′-untranslated regions contain short conserved elements complementary to parts of the lin-4 transcript.[13] These pioneering studies suggested that small antisense RNAs could bind and inhibit specific mRNAs that contain complementary sequences to the small RNA. A second small regulatory RNA called let-7 has been known for years yet was identified as an antisense RNA only in 2000.[14] The discovery of many regulatory 22-nucleotide RNAs led to the term *miRNA*.[15] With the expanded list of miRNA species came the need for better nomenclature.[16] Currently, each miRNA is assigned the prefix "miR," followed by a number that represents the order of naming. MiRNAs commonly are preceded by 3 letters that denote the organism (eg, "hsa-miR-121" is the human miR-121 and "mmu-miR-121" is the mouse version). Additional information on the miRNA naming system and miRNA sequences can be found in miRNA database such as miRBase.[8]

MiRNA biogenesis

MiRNAs are encoded in the genome and are typically transcribed as long primary miRNA (pri-miRNA) by RNA polymerase II[17] before undergoing a multistep process that leads to mature miRNAs (Figure 1). The pri-miRNA contains partially self-complementary regions that fold back and form a hairpin structure, which harbors the mature miRNA sequence. In the nucleus, the hairpin structure (65-80 nucleotides long) is released from the pri-miRNA by endonucleolytic cleavage executed by the