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The functions and applications of extracellular vesicles miRNAs in adverse pregnancy

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Abstract: During pregnancy, the maintenance of maternal health and fetal development is finely regulated by various factors. Adverse pregnancy can lead to pregnancy complications, premature birth, malformations, and congenital diseases in the fetus. Early detection and treatment of maternal-fetal diseases are crucial. Extracellular vesicles miRNAs serve as a means of communication between the mother and the fetus during pregnancy, undergoing significant changes in secretion and types during disease states, and playing an important regulatory role in the occurrence and development of diseases. Therefore, in pathologic conditions, extracellular vesicles miRNAs may mediate maternal-fetal communication, potentially leading to abnormal fetal development, and serve as early detection biomarkers for fetal diseases. Modified extracellular vesicles miRNAs can also be used as novel drugs for treating intrauterine diseases.

Keywords: extracellular vesicles; miRNAs; adverse pregnancy; intrauterine disease

1. Introduction

Maintaining a healthy pregnancy is of great significance for embryonic development and offspring health. Adverse pregnancy not only affects the health of pregnant women but also leads to abnormal fetal development. For example, pregnancy complications such as

preeclampsia, gestational hypertension, and gestational diabetes can result in premature birth, malformations, congenital heart diseases, and neural tube defects [1–4].

Currently, the detection of pregnancy-related diseases and fetal developmental disorders primarily relies on routine blood tests and ultrasound examinations. Limitations in the detection methods may result in missed diagnoses during the optimal period or irreversible outcomes. Therefore, there is a need for more sensitive novel biomarkers for early diagnosis [5,6].

There are various substances in the maternal peripheral blood that reflect the health status of both the mother and the fetus during pregnancy, including blood glucose [7], hormones [8,9], free fatty acids [10,11], and inflammatory factors [12,13], which are closely related to maternal and fetal health. In recent years, extracellular miRNAs, especially those encapsulated in extracellular vesicles, have attracted interest in the scientific community due to their involvement in various physiological and pathological processes. New research indicates that extracellular vesicle-derived miRNAs from maternal cells play a role in pregnancy processes, including implantation, embryonic development, and maintenance of the maternal immune microenvironment [14–17]. Under pathological conditions, maternal-derived extracellular vesicle miRNAs possess the characteristics of "signal carriers" that can be transmitted from the mother to offspring and may be associated with infant diseases [18]. These studies strongly suggest the crucial role of maternal extracellular miRNAs in offspring health.

This review will focus on extracellular vesicles miRNAs during adverse pregnancy and explore the new mechanism by which maternal-derived extracellular vesicle miRNAs can cross the placental barrier and potentially lead to abnormal embryonic development. Special attention will be given to the potential value of extracellular vesicle miRNAs as specific biomarkers for fetal diseases and as therapeutic drugs for intrauterine diseases.

2. Methods

As of November 14, 2023, a comprehensive search was performed in the PubMed database to retrieve relevant publications. The search terms comprised "extracellular vesicles miRNA," "obese pregnant offspring," and "extracellular vesicles maternal-fetal communication." The selection of these publications was based on their status as original articles and reviews specifically addressing the influence of maternal extracellular vesicles miRNA on the intrauterine development of fetuses under obese conditions. To provide a detailed overview of our search methodology, we organized the information in Table 1.

Table 1. Summary of the search strategy.

Items	Specification
Date of search	December 31, 2023
Databases and other sources searched	Pubmed
Search terms	Extracellular vesicles miRNA, obese pregnant offspring, extracellular vesicles maternal-fetal communication

Table 1. *Cont.*

Items	Specification
Timeframe	Papers published before November 14, 2023
Inclusion and exclusion criteria	Inclusion criteria: Focus on original articles and reviews in English about extracellular vesicles miRNA involved in adverse pregnancy and intrauterine development of fetuses. Exclusion criteria: Published in a language other than English.

3. Extracellular vesicles miRNAs in adverse pregnancy

In order to coordinate and respond to biological signals, extracellular vesicles miRNA is secreted into the extracellular space and delivers specific information to recipient cells. During pregnancy, the presence of blood-derived EVs undergoes dynamic changes, reflecting the health status of the mother-fetus unit and embryonic development. Studies have found that during pregnancy, extracellular vesicles miRNA can cross the placental barrier and be delivered from the mother to the fetus, making them a potential mechanism for studying fetal diseases and potential biomarkers for prediction and treatment.

3.1. Characteristics of extracellular miRNAs

With the completion of the Human Genome Project and the beginning of the post-genome era, there has been an upsurge in non-coding RNA research. miRNA is a class of short non-coding RNAs with a length of nucleotides 20–22. It negatively regulates most genes in cells by recruiting RNA-mediated silencing complex (RISC complex) and complementary pairing with target gene [19].

The generation of classical miRNAs begins with the generation of pri-miRNA transcripts, which are usually more than 1 kb in length, contain local stem-ring structures, and contain mature miRNA sequences. Endonuclease Ⅲ Drosha releases hairpin RNA (pre-miRNA) with a length of about 65 nucleotides by clipping the stem ring site to initiate the maturation process [20]. The export protein EXP5 can form a transport complex with the pre-miRNA and the GTP-binding nuclear protein RAN GTP. After translocation through the nuclear pore complex [21], the pre-miRNA is transported to the cytoplasm [22], where it is cut near the end ring by Dicer to form small double-stranded RNA. The double stranded miRNA is loaded into the Ago protein to form the RISC complex with the help of the HSP70/HSP90 chaperone. Among them, only one strand of double-stranded miRNA can be retained in the Ago2 protein and statically form the RISC complex, which is called the guide chain, and is also commonly referred to as miRNA.

Because the presence of RNA enzymes in the circulatory system can lead to the degradation of miRNAs, early studies believed that miRNAs can only play a role in the cell. Research in our lab in 2010 found that miRNAs can be stably present in the serum and plasma

of humans or other animals, including rats, mice, cattle and horses [23]. MiRNAs are encapsulated in EVs that resist RNase degradation and can be carried into target cells or tissues, where they combine with the untranslated region (UTR) of the target gene to induce post-transcriptional inhibition [24].

3.2. Composition and mode of action of extracellular vesicles

Extracellular vesicles (EVs) are membrane-derived vesicles secreted by cells into the extracellular space and play an important role in cell- cell communication [25]. EVs can be produced by almost all cell types in mammals, and can be stably present in blood, urine, milk, saliva and other body fluids [26–29]. Among them, extracellular vesicles are nanoscale extracellular microvesicles (sEV) with a diameter of 50–150 nm, which consist of the multivesicular body (MVB) formed from the inner depression of the secondary endosomal membrane, and MVBs can release ILV into the extracellular space by fusion with the cell membrane. EVs, as nanoparticles produced by the body itself, have immune tolerance, stable existence in the circulatory system and the ability to cross biological barriers (blood-brain barrier and placental barrier) to reach distal organs [5,30].

The composition of EVs has been extensively studied, and the membrane structure of EVs is characterized by high concentrations of cholesterol, sphingomyelin, and ceramides. Also included are unconventional phospholipids not present in other cell membranes, namely lysophosphatidic acids, which contribute to the accumulation of cholesterol [31]. Among the membrane proteins of EVs, quaternary transmembrane proteins and other transmembrane proteins make up the functional membrane structure. In addition, EVs can selectively load proteins, lipids, and non-coding RNA and DNA sequences [32].

After being released into the extracellular space by cells, EVs can reach the target cells through the circulatory system, and bind to the surface of the target cell membrane to activate membrane surface receptors and signal transduction, so that EVs can be internalized by endocytosis or fusing with the target cells [33], and deliver the contents such as miRNA to the target cells to perform functions, thus affecting the physiological and pathological state of the target cells [34]. After extracellular vesicle miRNA enters the target cell, it inhibits the expression of target genes at the post-transcriptional level, thereby affecting the activation/suppression [35,36], invasion/migration [37,38], autophagy [39], angiogenesis and endoplasmic reticulum stress [40] in the target cell.

3.3. Disease-related EVs miRNA changes

After extracellular vesicles miRNA enters target cells, it can regulate the tumor microenvironment [41], improve tumor growth [42], invasion [43], and metastasis. Extracellular vesicles can regulate angiogenesis in tumors by delivering miRNA [44], such as extracellular vesicles containing miR-23a inducing angiogenesis through targeting SIRT1 in endothelial cells [45]. The total amount of extracellular vesicles produced by multiple myeloma cells (HR-MM cells) increases, and the level of miR-135b is significantly upregulated. Upregulated miR-135b directly inhibits hypoxia-inducible factor 1 (FIH-1) and

enhances angiogenesis under hypoxic conditions [46]. MiRNA in extracellular vesicles can mediate immune evasion of tumor cells, thereby enhancing tumor cell survival and proliferation. Our laboratory also found in 2014 that extracellular vesicles secreted by tumor cells are enriched with miR-214, which is delivered to recipient T cells and promotes the expansion of Treg by suppressing PTEN expression, thereby contributing to immune evasion and tumor growth [47]. Christina Grange *et al.* demonstrated that extracellular vesicles derived from CD105-positive human renal cancer stem cells are rich in miR-151, miR-29a, and miR-650, which are associated with tumor invasion and metastasis [48]. Tumor cells can also use extracellular vesicles miRNA to sequester or expel drugs, promoting drug resistance [49–51].

The number of circulating EVs increases in metabolic diseases such as insulin resistance, diabetes, and nonalcoholic fatty liver disease [52–54]. Metabolic diseases has been shown in both clinical trails and rodent studies to lead to qualitative differences in protein and RNA in EVs. In a study that included 219 patients with extracellular vesicles miRNA expression profiles, it was found that patients with type 2 diabetes, hypertension, and high cholesterol had different EV miRNA expression profiles [55]. This may be related to changes in obesity-related metabolic tissue.

Adipose tissue is also an active endocrine organ that influences the body's state of health by secreting EVs. Rodent studies have also found that EV-mediated signaling between adipocytes and macrophages promotes the development of insulin resistance in spontaneously obese mice with leptin knockout [56]. In 2017, Ying ,W.et al. published a paper in Cell, which reported miR-155 levels are significantly elevated in macrophage derived EVs in adipose tissue of obese mice, and can enter fat, liver, and muscle cells, impairs insulin sensitivity in normal mice by targeting PPAR-γ [57]. Our laboratory study found that obesity leads to increased levels of miR-222 in EVs derived from white adipose tissue, which promotes obesity-related insulin resistance by inhibiting IRS1 expression in liver and skeletal muscle [58]. It was also found that the expression levels of miR-122, miR-192, miR-27a-3p and miR-27b-3p in circulating EVs of obese mice induced by high fat diet increased, which promoted insulin resistance of normal mice by affecting the expression of PPAR-α in white adipose tissue [59]. Skeletal muscle and pancreas are also major organs of insulin resistance. Our laboratory found that miR-29s expression was significantly elevated in EVs secreted by islets and can enter the liver, promoting insulin resistance by targeting p85α protein [60]. After exercise, extracellular vesicles derived from muscle carry specific miRNA to downregulate the expression of FOXO1 protein in the liver, improving insulin sensitivity [61].

3.4. EVs deliver miRNA across the placenta

3.4.1. Discovery of EVs mediating mother-to-child communication

EVs miRNAs can transmit maternal-fetal information across the placenta (Figure 1). In 2015, our laboratory found for the first time that exogenous plant miRNAs could be detected in human cord blood and amniotic fluid. By administering exogenous mature miRNAs from influenza virus and honeysuckle soup rich in miR2911 to pregnant mice by gavage, it was found that small non-coding RNAs could be absorbed through diet and delivered to fetal

liver. In order to further determine how exogenous miRNA enters the fetus, the presence form of miR2911 in the plasma after gavage was detected. The results showed that there was no significant change in plasma with EVs removed, while miR2911 was significantly elevated in EVs, suggesting that circulating miR2911 mainly exists in EVs, and its transplacental delivery may be mediated by EVs. To further demonstrate that transplacental small non-coding RNAs are mediated by EVs, siRNA of Alpha-fetoprotein (AFP) encapsulated in EVs was injected into the tail vein and transplacental delivery into the fetus was found to regulate the expression of AFP in the fetal liver. This suggests that EV-coated siRNA can cross the placenta and be delivered into the fetus, and has biological functions [18].

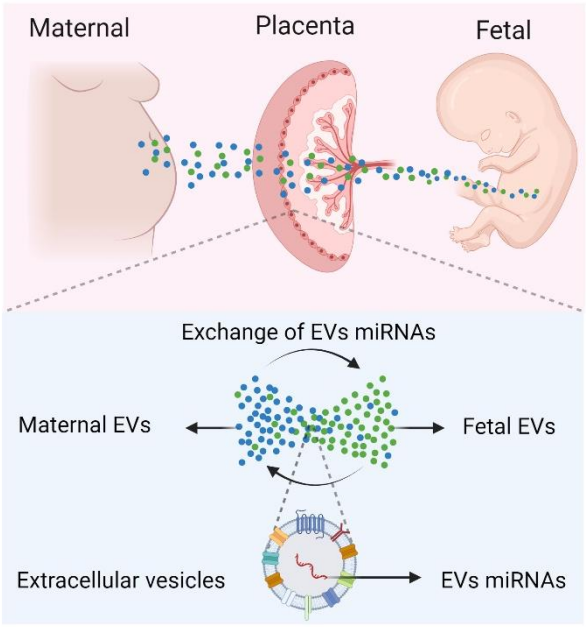

Figure 1. EVs miRNA mediate mother-to-child communication (Figure generated using BioRender).

Since then, more and more studies have found that EVs (mainly sEV) are important carriers of information exchange between mother and child. Ramkumar Menon's study found that EVs in fetal amniotic fluid can pass through the placenta and enter the maternal circulatory system. The fluorescent dye DiR labeled EVs were injected into the amniotic cavity of pregnant mice to detect their migration by imaging. After injection of DiR labeled extracellular vesicles at day 17 of gestation, they could be observed in maternal placenta and uterus as well as maternal plasma and kidneys at day 18 of gestation [62].

The use of cyclic recombinase (Cre) -dependent dual fluorescence reporting in transgenic mice demonstrated that EVs are carriers of mother-to-child information exchange during gestation. The mouse had tomato red fluorescent protein and EGFP green fluorescent protein, but under normal circumstances, only tomato was expressed, tomato was excised and EGFP could be expressed in the presence of Cre. When wild-type female mice mate with the transgenic mice, the fetus expresses tomato. The EVs isolated from maternal plasma are found to be tomato positive by pearl-coupled flow cytometry, and the fluorescence signal of tomato can be detected in the uterus and cervix of the mother, indicating that the EVs of the

fetus can enter the maternal circulation. In order to determine whether the EVs in the maternal circulation can enter the fetal body, the EVs containing Cre are injected into the pregnant mice through the tail vein. The results show that EGFP is expressed in the fetal placenta and fetal membrane, indicating that the fetus can ingest the maternal EVs and express EGFP under the action of Cre [63].

3.4.2. Effects of maternal EVs on fetal growth and development

Shi et al found that EVs in diabetic female dams mothers could lead to fetal heart development defects. Mice induced diabetes by STZ injection resulted in lower fertility, higher rates of fetal mortality and teratogenesis, and fetal heart development defects. Compared with normal mice, the diabetic pregnant mice showed significant changes in the levels of miRNAs in the EVs, including miR-133, miR-99, miR-23, and miR-30, which play a role in heart development. Further studies showed that the EVs of diabetic pregnant mice could enter the fetal heart after being labeled with fluorescent dye, resulting in cardiac development defects [64]. In line with these findings, Yunnan Liu *et al.* found that EVs derived from visceral adipose tissue in obese mothers increase the risk of fetal heart failure. Abnormal fetal heart development, accompanied by placental inflammation, was found in female mice induced by high fat diet containing 60% fat. Confocal microscopy showed that maternal adipose tissue-derived EVs could enter the fetal heart through the placental barrier, and obese murine-derived EVs increased the number of stillbirths and ROS levels in fetal hearts. This process may be mediated by miR-19b in the EVs. Electroporation loading of miR-19b into the adipose tissue-derived EVs of obese pregnant mice reduces the proinflammatory effect of the adipose tissue-derived EVs of obese pregnant mice [65].

3.4.3. The maternal sEV miRNAs have sex-specific effects on offspring

EV miRNAs play an important biological role in regulating early embryo-maternal interactions. For instance, mouse oviductal EVs can transfer miR-34c-5p to sperm heads and promote the first cleavage of fertilized eggs [66]. Oviductal EVs fuse with canine oocyte cells and release miRNA, promoting oocyte maturation and follicle growth [67]. During the embryo implantation process, EVs secreted by human endometrial epithelial cells increase has-miR-30d, which has been shown to promote embryo adhesion in mouse models [68]. Additionally, EV miR-34c-5p derived from mouse endometrium is differentially enriched at different developmental stages and affects embryo implantation by targeting GAS1 [69]

The influence of maternal miRNAs on offspring also exhibits sex-specificity. Researchers such as Alon Chen have found that susceptibility to anorexia in mice may originate during the intrauterine period, making female offspring more susceptible to metabolic challenges during puberty. Susceptibility to anorexia is associated with the endogenous levels of placental miR-340, with overexpression significantly increasing susceptibility to anorexia [70]. Human studies have shown that the expression profile of maternal serum miRNAs during fetal growth restriction exhibits sex-specific patterns. When female fetal growth is restricted, miR-28-5p is significantly reduced ($p < 0.01$); when male

fetal growth is restricted, miR-301a-3p is reduced ($p < 0.05$); and regardless of fetal sex, miR-454-3p is reduced during fetal growth restriction pregnancies. Additionally, exposure to maternal obesity in utero leads to gender-specific changes in miRNA and target gene expression in the fetal liver [71]. In pregnancies of obese mothers, the expression of has-miR-885-5p, has-miR-199-3p, has-miR-503-5p, has-miR-1268a, and has-miR-7-1-3p are elevated in the amniotic fluid, and these miRNAs also significantly increase in female fetal liver cells, leading to lower expression of the target genes *Abca1*, *Pak4*, and *Insr*. In male fetuses, there is no significant change in the above miRNAs, but there is an increase in the expression of the target genes [72]. The inflammatory intrauterine environment associated with maternal obesity induces an NFκB1-mediated increase of miR-210 in a gender-dependent manner, suppressing mitochondrial respiration and placental dysfunction in female fetuses [73].

3.5. Application of EVs miRNA in pregnancy

Extracellular vesicles also play a role as markers and therapeutics in many diseases. As mentioned earlier, in disease states, EVs accumulate in body fluids, especially in plasma, making them important for minimally invasive liquid biopsies and their potential for longitudinal sampling and tracking of disease progression. Therefore, by analyzing the presence of EVs in body fluids and their specific components, including DNA and miRNA, EVs can be used as important biomarkers for the diagnosis and treatment of pregnancy diseases.

3.5.1. EVs miRNA as biomarkers

The EVs miRNA as a biomarker demonstrates high sensitivity and specificity [74–78]. The research on large-scale EV-miRNA expression profile generated by high-throughput technology is increasing, which lays the foundation for EV-miRNA to be called a more systematic and unbiased biomarker.

Preeclampsia is one of the major causes of maternal and neonatal death and morbidity. There are significant differences in plasma EVs miRNAs between normal pregnancy and preeclampsia (Figure 2). In a study involving 34 healthy controls and 64 pregnant women affected by different types of hypertension (chronic hypertension: N = 16; gestational hypertension: N = 14; moderate preeclampsia: N = 15; severe preeclampsia: N = 19), it was found that the total miRNA content (N: 0.26 ng/μL; GHT: 0.28 ng/μL; CHT: 0.30 ng/μL; mPE: 0.83 ng/μL; sPE: 1.57 ng/μL) and hsa-miR-210 concentration(N: 0.13; CHT: 0.17; GHT: 0.23; mPE: 0.29; sPE: 0.37) in plasma EVs of patients with gestational hypertension were significantly increased, and were positively correlated with the severity of gestational hypertension [79]. Therefore, peripheral blood EV miRNA is expected to be an early detection marker to identify different types of pregnancy-induced hypertension.

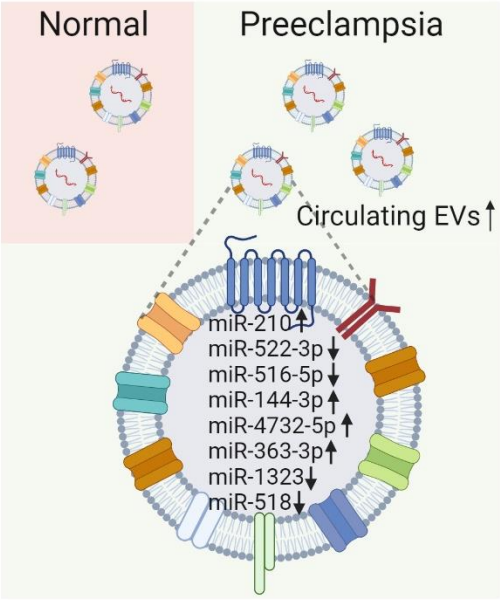

Figure 2. Comparative EVs miRNA of normal pregnancy and preeclampsia (Figure generated using BioRender).

Gestational diabetes mellitus (GDM) is also one of the most common complications of pregnancy. By sampling prospective patients at 11–14 weeks, 22-24 weeks and 32-36 weeks of gestation, EV concentration increased during both normal (N = 13) and GDM (N = 7) gestation. However, the increase of GDM was significantly larger (compared with normal gestation, 2.2-fold, 1.5-fold, and 1.8-fold) increases for each gestational age, respectively [80]. Compared with normal pregnancy (N = 64), miR-122-5p (mean, 1.42 ± 0.17 vs 0.98 ± 0.09; P = 0.01); miR-132-3p (mean, 1.30 ± 0.12 vs 0.94 ± 0.08; P = 0.03); miR-136-5p (mean, 1.48 ± 0.24 vs 0.99 ± 0.10; P = 0.03); miR-182-3p (mean, 1.51 ± 0.20 vs 0.99 ± 0.11; P = 0.01); miR-29a-3p (mean, 1.43 ± 0.22 vs 0.97 ± 0.09; P = 0.03); miR-29b-3p (mean, 1.40 ± 0.22 vs 0.95 ± 0.10; P = 0.04); miR-210-3p (mean, 1.67 ± 0.39 vs 0.97 ± 0.08; P = 0.02); miR-342-3p (mean, 1.50 ± 0.18 vs 0.95 ± 0.11; P = 0.008); miR-520h (mean, 1.2 ± 0.2 vs 0.80 ± 0.07; P = 0.03) and miR-1323 (mean, 1.54 ± 0.26 vs 0.97 ± 0.13; P = 0.03) were present in serum EVs in GDM pregnancies (N = 23) were significantly increased [81].

Giving birth before 37 weeks of gestation is considered premature, and preterm babies are at significantly increased risk of respiratory diseases, cerebral palsy and other conditions. The expression of miR-302b, miR-1253 and miR-548 (Data mean +95% CI, $p < 0.0001$) in plasma EVs was decreased in the preterm group (N = 9), while the expression of miR-223 ($P < 0.001$; Control, 1731 ± 223 vs. Preterm, 2945 ± 304) was significantly increased compared with the control group (N = 7) with full-term delivery. Therefore, circulating miRNA may be used as a marker to identify preterm birth [82].

In addition, in plasma samples from pregnant women with Down syndrome, Circulation hsa-miR-3156 (2.57-fold) , hsa-miR-99a (4.20-fold), hsa-miR-15a (1.78-fold), hsa-let-7d (1.73-fold), hsa-miR-142 (1.61-fold), hsa-miR-23a (1.63-fold), hsa-miR-199 (1.59-fold), hsa-miR-191 (1.91-fold) upregulation, hsa-miR-1290 (1.81-fold), hsa-miR-1915 (1.85-fold), hsa-miR30e (1.28-fold), hsa-miR-1260 (1.43-fold), hsa-miR-483 (1.34-fold), hsa-miR-548

(1.52-fold), and hsa-miR-590 (1.43-fold) are down-regulated. The genes regulated by these differential miRNAs are involved in fetal central nervous system development, congenital abnormalities and heart defects [83–85].

A study involving 30 pregnant women with only one fetus diagnosed with coronary heart disease were selected as cases, while another 30 pregnant women with only one normal fetus were defined as the control group, revealed that circulating miR-19b (2.22-fold), miR-22 (2.74-fold), miR-29c (3.38-fold), and miR-375 (3.03-fold) were significantly elevated in pregnant women with congenital heart disease [86]. These studies suggest that maternal plasma EVs and extracellular miRNAs can be used as markers for early detection of maternal and fetal diseases during pregnancy (Table 2).

Table 2. Extracellular vesicles secreted in different body fluids and their potential markers.

Pregnancy complication	Potential markers	Source	References
Preeclampsia	hsa-miR-210; hsa-miR-522-3p/hsa-miR-4732-5p; hsa-miR-516a-5p/hsa-miR-144-3p; hsa-miR-27b-3p/ hsa-let-7b-5p.	Plasma	[9,87]
GDM	hsa-miR–122-5p; hsa-miR–132-3p hsa-miR–136-5p; hsa-miR–182-3p; hsa-miR–29a-3p; hsa-miR–29b-3p; hsa-miR–210-3p; hsa-miR–342-3p; hsa-miR-520h; hsa-miR-1323.	Serum	[81]
Preterm birth	hsa-miR-302b; hsa-miR-1253; hsa-miR-548; hsa-miR-223.	Plasma	[82]
Down syndrome	hsa-miR-3156；hsa-miR-99a; hsa-miR-15a；hsa-let-7d; hsa-miR-142；hsa-miR-23; hsa-miR-199；hsa-miR-191; hsa-miR-1290；hsa-miR-1915; hsa-miR30e；hsa-miR-1260; hsa-miR-483；hsa-miR-548; and hsa-miR-590.	Plasma	[83]
Congenital heart defects	hsa-miR-19b; hsa-miR-22; hsa-miR-29c; hsa-miR-375.	Plasma	[86]

3.5.2. EVs miRNAs as a therapeutic strategy

Due to the high stability, low toxicity, low immunogenicity of EVs in the circulatory system and their targeting to organs, tissues or cells, EVs themselves can be used as drugs or as drug delivery carriers [4,88–90]. Because EVs can cross the placental barrier and have the advantages of safety and non-invasive, they become a new choice for intrauterine delivery carriers, which has been verified in a mouse model.

EVs enriched by surface ligands promote the development of receptor-mediated tissue-targeted drug delivery. Ligands that have been genetically engineered to modify EVs can be

used to induce or inhibit signal transduction in target cells, or to target specific receptor cells. For example, the addition of Lamp2b may promote tumor targeting [91]. The placental CSA-binding peptide (plCSA-BP) developed by Zhang *et al.* 2018 can effectively increase targeted placental delivery [92]. Tumor homing pentapeptide CGKRK and Tumor homing cyclic peptide that target integrin receptors in the placental vascular system iRGD) modified liposomes have been used to deliver carboxyfluorescein and IGF-2 to improve fetal weight [93]. Liposomes modified with CNKGLRNK, a novel placental homing peptide targeting the placenta and uterine endothelium, can selectively deliver nitric oxide donors to the placenta and improve fetal growth restriction [94].

Based on the results that miRNAs in EVs effectively inhibit the expression of target genes, EV engineering has been developed for the delivery of specific miRNAs or siRNAs. For example, EVs modified with GE11 synthetic peptides can deliver let-7a to breast cancer cells and inhibit their growth [95]. EVs derived from mesenchymal stem cells can deliver miR-146b to rat gliomas and target EGFR [96]. These studies lay the foundation for the development of EVs as therapeutic agents.

Currently, there are two main ways to load active molecules for therapeutic use into EVs. One is to purify the EVs and then load them with therapeutic active molecules. The most widely used is to load small molecules such as siRNA or miRNAs into the EVs by electroporation technology. The other is to load therapeutic active molecules into EVs during vesicle biogenesis. For example, miRNAs is transfected into cells, and the natural transport mechanism is used to load miRNAs during the formation of EVs. Another way to load miRNAs into EVs is to use a viral packaging strategy.

Studies have shown that EV miRNAs are low in toxicity and immunogenicity. EVs derived from human HEK293T cells loaded with miRNAs and proteins were injected into C57BL/6 mice via caudal vein and intraperitoneally. After 3 weeks of continuous administration, blood was collected to evaluate hematology, blood chemistry, and immune markers. Splenic cells were evaluated for immunophenotyping and tissue was collected for histopathological examination. No signs of toxicity were observed [97]. However, the immunological and pharmacokinetic properties of EVs may be different due to the different parental cells from which they were derived, so further research is needed to evaluate the therapeutic effect, clarify toxicity and clearance, and whether there are long-term effects in the conduct of patent drug studies. In addition, the liver self-assembly technology developed by our laboratory in 2021 can secrete targeted modified exosomes to simultaneously wrap nucleic acid drugs for the treatment of diseases, which can effectively solve the problems such as the immunogenicity of source cells and production cost, and is expected to become a delivery method for developing new nucleic acid drugs for the treatment of Intrauterine disease [98–100].

4. Conclusion

EVs miRNAs is a mechanism of maternal-fetal information exchange during pregnancy. The content and types of EVs miRNAs change in the pathological conditions, and can be

delivered across the placenta to fetal mice, so it may affect fetal development. On the other hand, the timing and specific role of maternal EV miRNAs in fetal development remain to be determined. EVs miRNAs-mediated maternal-fetal communication may provide a unique strategy for intrauterine disease etiology, early detection and treatment.

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Conflicts of interests

The authors have no other conflicts of interest to declare.

Authors' contribution

Yujing Zhang designed framework and revised the manuscript. Huanhuan Shi and Lina Ma drafted the manuscript, Benben Sun and Huanhuan Shi contribute to discussions. All authors have read and approved the final manuscript.

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