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THE ROLE OF EXTRACELLULAR VESICLES IN PREECLAMPSIA

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ABSTRACT

Preeclampsia (PE) is a pregnancy-associated disease characterized by hypertension and proteinuria, and the leading cause of maternal and fetal mortality worldwide. PE is believed to be caused by alterations in feto-maternal communication. Since extracellular vesicles (EV) are crucial for cell-to-cell communication, their role in pregnancy and pregnancy-associated diseases has been a matter of active investigation. Recent evidence points to the role of maternal and placental EV in the regulation of angiogenesis, hormonal and immune mechanisms during pregnancy. This minireview focuses on how alterations in the levels of maternal and fetal EV may contribute to the pathogenesis of preeclampsia. A better understanding of feto-maternal EV crosstalk with cellular and molecular signaling pathways involved in fetal development may be crucial to early diagnosis and therapeutic interventions for PE.

Keywords: Preeclampsia, Extracellular vesicles, Inflammation, Biomarkers.

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INTRODUCTION

Preeclampsia (PE) is a pregnancy-related disease with a global incidence ranging between 5%-7%.[1] It is the major cause of adverse pregnancy outcomes, maternal and fetal mortality worldwide.[1,2] PE occurs usually after 20 weeks of gestation and is clinically diagnosed by the presence of an elevated blood pressure (BP) and proteinuria.[3] PE is classified as mild when the BP is ≥140/90 mmHg for at least two measurements, and proteinuria is ≥+1 (qualitatively detected in a random urine sample by dipstick) or ≥300 mg/24h.[4] Severe PE occurs when BP is ≥160/110 mmHg for at least two measurements concomitantly with heavy proteinuria (≥+3 by dipstick or ≥5 g/24h) and multi-organ vascular damage.[4]

PE is recognized as a complex web of abnormalities characterized by vascular endothelial damage caused by increased placental release of soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sENG), which are anti-angiogenic via inhibition of placental growth factor (PGF), vascular endothelial growth factor (VEGF), and endothelial nitric oxide-dependent vasodilation, all of which are necessary for placental angiogenesis and remodeling of uterine spiral arteries during the second half of pregnancy.[5-7]

Although previous studies have identified several PE risk factors, including elevated pre-pregnancy body mass index, smoking, pre-gestational diabetes mellitus and dyslipidemia[8-10], the underlying cellular and molecular pathogenesis is not well understood, thus making early diagnosis and therapeutic intervention for PE difficult. Recent studies, however, have shown that a group of biomolecules called extracellular vesicles (EV) released from the placenta may be crucial to understanding the cellular mechanisms of PE pathophysiology and the discovery of reliable predictive biomarkers of PE.[11]

EV are lipid membrane structures secreted by an array of cell types in the body and released into the extracellular space under normal physiological state and pathological conditions.[11,12] EV are vital for intercellular communication, interacting with adjacent and distant cells, and altering cells' biological functions upon delivery of their cargos composed of proteins, lipids and RNAs.[13,14]

Three major types of EV have been identified based on their size, shape and functions: these are microvesicles (microparticles), exosomes, and apoptotic bodies. Apoptotic bodies are remnants of the apoptotic process, and represent the largest EV with sizes ranging from 1000 to 5000 nm.[15] Microvesicles are the direct products of endocytosed vesicular materials; their size ranges from 100 to 1000 nm.[15,16] Exosomes, which are the smallest EV (40-120 nm) are formed from multivesicular bodies and released into the circulation via exocytosis.[17] Having overlapping sizes and being simultaneously present in biological fluids has been a challenge for the characterization of EV subtypes. However, the identification of cell specific EV components have offered some solutions. For instance, Arf6, CD81 and Annexin V are markers for microvesicles, exosomes and apoptotic bodies, respectively.[15,18]

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The search for reliable predictive and diagnostic markers of PE has benefitted from the characterization of EV. Changes in EV composition in the maternal blood circulation and placenta have been reported during pregnancy-associated diseases. Given the low invasiveness of sampling blood for their characterization and quantification, EV may be key to finding reliable predictive biomarkers for PE. This minireview provides an overview of the role of maternal and placental EV in preeclampsia and discusses their potential as predictive biomarkers of PE.

Feto-maternal interaction

In humans, after fertilization, the blastocyst encapsulated by the zona pellucida emerges within 4 to 6 weeks. This emergence initiates the communication between the embryonic and maternal cells to promote the growth of the embryo. The communication is facilitated by the trophoblasts which are the cells of the outer layer of a blastocyst. More specifically, the formation of the feto-maternal interface is promoted by the two layers of the trophoblasts called syncytiotrophoblast (SCT) and cytotrophoblast (CYTB). Having direct contact with the maternal blood, the syncytiotrophoblast supports the exchange of oxygen, nutrients, wastes and immune factors between the mother and fetus. The cytotrophoblast serves as anchor for chorionic villi and differentiates into another type of epithelial trophoblasts called extravillous trophoblasts (EVT). EVT further establishes the maternal-placental unit and strengthens the connections between the placenta and uterine wall by invading the decidualized uterus, which brings about the remodeling of maternal uterine spiral arteries.[19]

It is believed that uterine receptivity and consequently fetal growth is enhanced by the hormonal and immunological effects of the maternal-placental unit. For instance, a metabolite of estriol called 4-hydroxy catechol is known to mediate blastocyst implantation.[20] Also, EVT invasion evokes the expression of chemokines and their receptors, which are involved in immune regulation. The chemokine receptors, including CXCR4, CXCR7 and CXCL12, are crucial for the formation of the feto-maternal interface, the survival of trophoblastic cells, and they inhibit apoptosis.[21] The decreased expression of these chemokine receptors on the immune cells of women with PE has been reported.[21] It implies that any defect of the human feto-maternal interface arising from inadequate EVT invasion secondarily to chemokine receptor downregulation will impair the remodeling of uterine spiral arteries and cause abnormal placentation with clinical and biochemical features of PE.

Extracellular vesicles during pregnancy and PE

EV are deemed necessary for the establishment and continuance of pregnancy. Studies have shown that the feto-maternal communications are enhanced by the activities of several EV.[22] In particular, during embryo implantation, the migration of vascular smooth muscle cells, a necessary event for spiral artery remodeling, is promoted by EVT-derived exosomes, which underscores the importance of EV during early pregnancy.[23] Furthermore, the immunosuppressive action of placental EV helps to prevent the rejection of embryo implantation within the uterus by inhibiting the activation of maternal T lymphocytes, macrophages and natural killer cells.[24,25] This role has been attributed to the presence of UL 16 binding protein 5 (ULBP1-5), B7 homolog 3 protein (B7-H3) and human leukocyte antigen-G5 (HLA-G5) isoform on placenta-derived exosomes.[24-26]

Abnormal levels of circulating placental EV are associated with PE (Figure 1). Recent studies attributed these EV level anomalies to disruption of the placental milieu following hypoxia and oxidative stress, which is characteristic of impaired placentation accompanied by increased release of EV from the SCT as well as changes in EV contents and activity.[27-30] There is evidence that microvesicles from the SCT appear earlier during the first trimester, however, they appear at markedly higher levels in PE than in healthy pregnancies.[31] SCT-derived microvesicles attach to macrophages in maternal decidua and upon interaction with SCT-produced fibronectin, macrophages secrete elevated levels of proinflammatory cytokines.[32] In addition, SCT-derived microvesicles support the growth of T-helper-1 (Th-1) cells over that of Th-2 cells in PE resulting in the release of proinflammatory cytokines in the serum, including tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interferon-gamma (IFN-γ), which promote maternal vascular endothelial damage.[32-37] Hence, these EV promote chronic inflammation observed in PE, and the concomitant detection of EV and pro-inflammatory cytokines during the early stage of pregnancy would be invaluable in predicting PE.

Roles of EV anti-angiogenic factors in PE

Adequate angiogenesis is essential for the implantation and sustenance of conceptus in the early stage of gestation. PE is characterized by

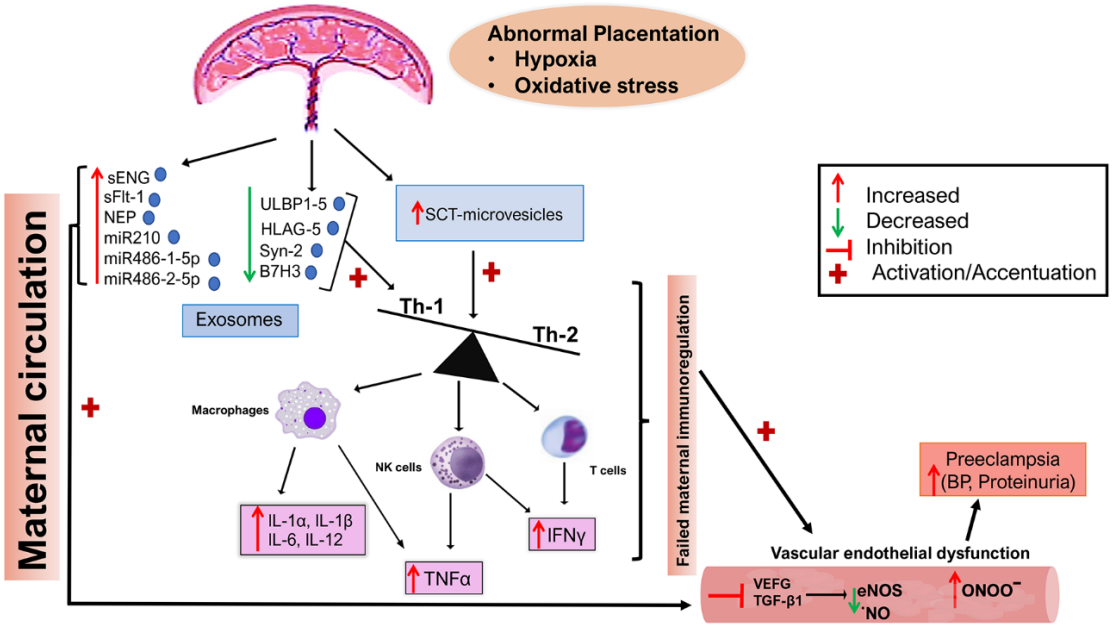

Figure 1: Dysregulated release of extracellular vesicles (EV) from placenta into maternal blood circulation during preeclampsia.

Inadequate trophoblast invasion and abnormal remodeling of spiral arteries during pregnancy cause abnormal placentation resulting in dysregulation of placental EVs. Under this condition, placental EV released into the maternal blood carry biomolecules that exacerbate maternal immunodysregulation and anti-angiogenesis leading to reduced nitric oxide production, elevated free radicals (peroxynitrite $ONOO^-$) and in turn multi-organ vascular endothelial dysfunction, hypertension and proteinuria, which characterize PE.

angiogenesis dysfunction which manifests by elevated levels of anti-angiogenic factors including sFlt-1 and sENG [38]. Pillay et al. reported that EVT-derived exosomes expressing sFlt-1 and sENG were markedly elevated in women with PE at a gestational age of less than 34 weeks[39].

Some of the commonly used biomarkers for PE are released by maternal and fetal EV especially those involved in the regulation of angiogenesis, which include VEGF, PGF, sENG, sFlt-1, tissue inhibitor of metallopeptidase 1 (TIMP1), plasminogen activator inhibitor type 1 (PAI1) and transforming growth factor beta-1 (TGF-β1).[6,40] Tan et al. determined these EV-related biomarkers in the plasma of PE patients by using cholera toxin B chain (CTB) and annexin V to isolate EV, and reported that the levels of interleukin-6, TIMP1 and sENG were increased in CTB vesicles, while tumor growth factor, PAI1 and S100b were increased in both CTB and annexin V vesicles.[40]

Similarly, it was reported that CTB-TIMP, annexin V-PAI1 (100% sensitivity, 78.6% specificity), but not maternal plasma TIMP or PAI1, predicted PE in a low-risk population at a mean gestational age of 7.3 ± 2.9 weeks before a formal diagnosis of PE was made.[41] This observation suggests that EV not only provide transport for these biomolecules associated with pregnancy and pregnancy-associated diseases, but they also facilitate their measurement within maternal blood, thus making EV potential predictor of PE.

Motta-Mejia et al. observed that SCT-derived EV from women with PE can repress endothelial nitric oxide synthase (eNOS) leading to vascular endothelial damage, elevated BP, and proteinuria.[42] Levels of SCT-derived EV expressing active neprilysin (NEP), an endopeptidase involved in promoting placental ischemia, were elevated in the blood of women with PE.[28] It was also reported that levels of placenta-derived exosomes carrying syncytin-2 (Sync-2), a protein crucial for the differentiation of CYTB and the inhibition of maternal immune response through PD-1/PDL and Fas-FasL pathways, are significantly decreased in women with PE.[43,44] Thus, NEP and Sync-2 may be useful biomarkers for the early detection of PE.

Roles of EV microRNAs in PE

A growing body of evidence indicates that healthy pregnancies and pregnancy-related diseases are influenced by the materials contained in EV. Studies

reported on the role of EV cargos in maintaining a balance between angiogenic and anti-angiogenic factors, the loss of which contributes to endothelial dysfunction and ischemic damage in pregnant women with PE.[45,46] In particular, microRNAs (miR) present in placenta-derived exosomes may modify the expression pattern of genes associated with angiogenic processes during pregnancy. For instance, miR-31, miR-150 and miR-125a, which were found in maternal EV, are involved in the initiation and promotion of angiogenic processes during pregnancy.[47-50] In contrast, placental exosome-derived anti-angiogenic miR-155, which has been shown to promote endothelial dysfunction by repressing eNOS expression, is increasingly released from PE placenta.[51]

There is evidence that the regulation of inflammatory responses in pregnant women is promoted by EV microRNAs. Matsubara et al. reported that miR-517a present in trophoblast-derived exosomes is involved in maternal immune response during pregnancy via regulation of Th-1/Th-2 balance and prevents the suppression of anti-inflammatory cytokines.[52] Meanwhile, certain EV microRNAs have been shown to be pro-inflammatory during PE, particularly placental exosome-derived miR-494 has been shown to inhibit macrophage polarization to M2 subtype through the suppression of uterine PGE2 secretion, which participates in maintaining the immune balance at the feto-maternal interface.[53] Placental exosome-derived miR-210 was upregulated in PE women and linked to promoting inflammation by downregulating the STAT6/IL-4 pathway.[54,55] Furthermore, hsa-miR-486-1-5-p and hsa-miR-486-2-5-p copies were enriched in placenta-derived exosomes of women with PE when compared to subjects without PE.[56]

Taken together, the detection of these exosome-derived microRNAs released from the placenta into the maternal blood circulation during the early stage of pregnancy could be useful for early detection of PE.

CONCLUSION

EV play a crucial role in the interactions between maternal and fetal tissues during pregnancy. Despite acknowledging the plethora of functions exhibited by EV and their cargos in placentation, angiogenesis and immunoregulation, the usefulness of EV for diagnostic purposes is hampered by the lack of standardized procedure for the identification of EV in clinical settings. Further studies on how feto-maternal EV crosstalk with cellular and molecular signaling pathways involved in fetal development would be critical for establishing precise and accurate methods of PE early detection.

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