Syncytiotrophoblast-derived extracellular products associated with preeclampsia

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ABSTRACT

The maternal-embryo-fetal interface is a site of exchange of biochemical and immune information. In a normal pregnancy, the syncytiotrophoblast releases different trophoblastic components including extracellular vesicles (EVs), microparticles (MPs), exosomes, and microRNA. These products exert local changes in the microenvironment and can impair different maternal functions. Placental exosomes can normally be detected in maternal blood from the first trimester of pregnancy and their levels increase until the end of gestation. The development of preeclampsia is partly related to abnormal secretion of these products and thus alteration of normal maternal mechanisms. Syncytiotrophoblastic MPs play an important role in the occurrence of thrombotic complications of pregnancy. Trophoblastic cells also release microRNA encapsulated within EVs or bound to Argonaute proteins, which are then passed onto the maternal circulation where they are highly stable. These proteins are specialized binding molecules that adapt to microRNA and participate in the silencing of genes and also interact with other proteins. Finally, the increased release of trophoblastic products causes alterations that contribute to maternal morbidity (preeclampsia) or the development of diseases in adult life in both the mother and newborn long after pregnancy has ended.

KEYWORDS

Exosomes, extracellular vesicles, microparticles, microRNAs, preeclampsia, syncytiotrophoblast, hypertension, placenta.

Introduction

The syncytiotrophoblast is the maternal-fetal interface for all types of interchange. The communication that takes place between the embryo and the mother includes biochemical and immune adjustments. The trophoblast also releases debris that may expand maternal-fetal relationships outside the uterus, and produce profound changes in all maternal organs and systems. All trophoblastic debris may consist of products of cell death that can induce changes and disruption of maternal cells [1]. The syncytiotrophoblast and the placenta continuously transfer extracellular vesicles (EVs) into the maternal circulation, including several molecules such as hormones, proteins, RNA and DNA. Thus, EVs of placental origin have been identified in the maternal circulation throughout normal pregnancy, and can be categorized according to their size as microvesicles (in the range of 150-1000 nm) and exosomes (in the range of 40-120 nm). Human maternal exosomes induce alterations in the receptor tissue and stimulate endothelial cells to release cytokines [2].

Preeclampsia/eclampsia is a multisystem disorder that occurs in 5% of all pregnancies and clinically manifests during the second half of gestation. Worldwide, it is still the leading cause of maternal and perinatal morbi-mortality. To date, there are no effective preventive or therapeutic interventions for this entity and several metabolic derangements associated with the disorder cannot be medically controlled. In general, preeclampsia has been linked to a failure of different inhibitor systems that control the tolerance of the embryo [3]. In addition, impaired placental implantation, hypoxia, endothelial dysfunction, and systemic inflammation are also thought to have a role in the pathogenesis of preeclampsia [4]. Despite the identification of these factors, improving clinical outcomes of preeclampsia has not been feasible. Moreover, many changes induced by preeclampsia can also have future negative health consequences in the mother and the fetus.

Syncytiotrophoblast, cell-free DNA and messenger RNA shedding is increased in pregnancies complicated by preeclampsia, as compared with normal pregnancies, and related to placental ischemia, reperfusion and oxidative stress [5]. Deportation of the above-mentioned material was initially demon-

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Abbreviations
ASA: acetylsalicylic acid; **DNA:** deoxyribonucleic acid; **EVs:** extracellular vesicles; **MPs:** microparticles; **RNA:** ribonucleic acid

strated in women with preeclampsia/eclampsia and in those with ectopic pregnancies, but it was later suggested that this process normally occurred in all pregnancies [6]. Increased syncytial deported fragments have been found to consist of multinucleated trophoblast fragments, anucleated cytoplasmic fragments, microparticles (MPs), and nanovesicles [7].

Trophoblast extracellular vesicles and microparticles

Extracellular vesicles (also known as microvesicles) are released by different cells and are present in the blood under normal healthy conditions. However, their concentrations are increased in different diseases. Their content includes proteins, growth and apoptotic factors, DNA fragments, and messenger RNA, as well as microRNA; therefore, they may function as regulators of cell-cell communication and mediators of cell signaling during multiple biological processes [8]. EVs is a non-specific term used to refer to membrane-bound vesicles of different origins, with diameters ranging from nanometers to micrometers [9].

Syncytiotrophoblast cells release EVs containing proteins, RNA and lipids. Proteomic studies have shown that EVs contain three proteins in common: albumin, fibronectin-1 and plasminogen activator-1. These are present in variable proportions according to the type of syncytiotrophoblast cell [10]. Syncytiotrophoblast EVs may impair the physiological functions of maternal organs [11]. In experimental conditions, mice develop preeclampsia (hypertension and proteinuria) only when purified EVs are injected, showing vasoconstriction and disruption of the endothelial integrity. Conversely, enhancing the clearance of EVs prevented the development of the preeclampsia [12]. Women with preeclampsia display an increased release of placental MVs and nanovesicles [13].

Syncytiotrophoblast EVs contribute to the excessive pro-inflammatory and procoagulant state observed in preeclamptic women. In addition, the protein composition of EVs from preeclamptic women differs from that found in normal pregnancies. Baig *et al*. [14] have identified over 400 proteins in samples of EVs, finding over 25 different proteins expressed in preeclampsia as compared with normal pregnancies. These different proteins include integrins, annexins and histones that could be relevant in the pathophysiology of preeclampsia [14]. Proteomic analysis of syncytiotrophoblast EVs from patients with early-onset severe preeclampsia characteristically shows up-regulation of 122 proteins and down-regulation of 72 proteins. In addition, there were four differentially expressed proteins related to inflammation, coagulation or immunoregulation [15].

Microparticles cause thrombus formation that has pro-inflammatory effects and causes endothelial dysfunction. Syncytiotrophoblastic MPs are increased in pregnant women as compared with healthy non-pregnant women and may play an important role in the occurrence of thrombotic complications of pregnancy. Some studies have reported the presence of MPs in maternal blood, finding this to be associated with an increased risk of miscarriage,[16] and in umbilical circulation in cases of preeclampsia [17]. These MPs are involved in coagulation, inflammation and transportation [18]. MPs may also participate in the hypercoagulable state of both normal pregnancies and pregnancies with placenta-related complications. In normotensive pregnancies, circulating MPs increase throughout the trimesters of gestation, with the highest levels being found at the end of pregnancy [19]. The excess of syncytiotrophoblastic MPs can be aggravated by hypoxia [13]. As measured by flow cytometry, women with preeclampsia display higher circulating total MP levels immediately after delivery, and lower platelet-derived MP levels as compared with normotensive women [17].

Exosomes

Exosomes are a specific population of EVs, which are highly stable and are present from the early stages of normal pregnancy in maternal fluids including urine and blood. Their maternal concentrations increase during the first three months of pregnancy [20]. Exosome levels are higher in women whose pregnancies are complicated by preeclampsia or gestational diabetes than in pregnant women without complications [21].

Exosomes are specifically defined by endosomal biogenesis and by particle size (40-120 nm) and density (1.13-1.19 g/mL[-1]). Exosomes regulate the activities of both proximal and distal target cells, including translational activity, angiogenesis, proliferation, metabolism, and apoptosis. Exosomes are specifically packed with signaling molecules (including protein, messenger RNA, microRNA, and non-coding RNA) and they are released by exocytosis into biofluid compartments [9]. Plasma exosome levels are increased in women with preeclampsia as compared with women experiencing normal pregnancies (normal, 3.88±0.23 versus preeclampsia, $6.14\pm1.45\times10^9$ total exosomes/mL) [22]. Equally, plasma exosomal protein levels are increased as a result of the divergence in exosomal content in pathological pregnancies [23]. In addition, there is a correlation between circulating placental exosome concentrations and maternal body mass index [24].

Chang *et al*. [23] postulated that exosomes may alter vascular development through the secretion of anti-angiogenic factors. Thus, preeclampsia is associated with exosomes that express soluble fms-like tyrosine kinase-1 and soluble endoglin. These authors were also able to collect exosomes containing high levels of soluble fms-like tyrosine kinase-1 and soluble endoglin by overexpressing them in human embryonic kidney 293 cells. Furthermore, exosomes produce the transfer of soluble fms-like tyrosine kinase-1 and soluble endoglin that can cause endothelial cell damage [23].

MicroRNAs and preeclampsia

MicroRNAs may play a key role in maternal-placental-fetal communication [25]. In preeclampsia, placental dysfunction leads to aberrant extracellular microRNA secretion. Hsa-miR-210 is a hypoxia-sensitive microRNA found to be upregulated in preeclampsia; however, it is unknown whether it is the cause or the consequence of the disorder [26]. MicroRNAs are short non-coding RNAs that regulate gene expression and

affect cell development, proliferation, differentiation and function. Women with preeclampsia display altered microRNA expression, although the role of microRNAs in the pathogenesis is unclear. Placental microRNA-210 is significantly increased in women with preeclampsia and in human trophoblasts during hypoxia [4]. However, the role of this product is not clear.

Trophoblastic microRNAs are passed into the maternal circulation, where they are highly stable, being encapsulated inside EVs, like exosomes, or bound to Argonaute proteins. These Argonaute proteins are specialized binding molecules that adapt to microRNA and participate in the silencing of genes and the interaction with other proteins [27]. Several microRNAs participate in hypertensive disorders of pregnancy. In preeclampsia, especially in its severe form, the most common associated microRNA is has-mir-210, which has shown higher observed levels in this disorder as compared with other hypertensive disorders of pregnancy [26,28].

Zhong *et al*. [29] compared the differential expression of plasma microRNAs in normal and preeclampsia pregnancies using quantitative polymerase chain reaction. They demonstrated that three plasma microRNAs were upregulated in women with preeclampsia (hsa-miR-1304-5p, hsa-miR-320a and hsa-miR-5002-5p) and were involved in cell proliferation. In addition, there were 26 downregulated microRNAs, as identified by microarray methods. Some of these microRNAs participate in the immune regulation and development of different pathological conditions, such as cancer, Alzheimer's disease and psoriasis [29,30].

Exosomes in the clinical scenario of preeclampsia

Different observational and randomized trials have shown the benefits of acetylsalicylic acid (ASA) for the prevention of preeclampsia [31]. When syncytiotrophoblast cells are cultured in the presence of different ASA concentrations and various oxygen tensions, exosome release is decreased with lower oxygen tensions and higher ASA concentrations. Hence, it seems that ASA may affect exosome release depending on oxygen tension [32]. There have been attempts to predict the risk of preeclampsia by measuring placental specific C19MC microRNAs in plasma exosomes during the first trimester of pregnancy [33]. Indeed, preliminary studies suggest that some exosome-expressed microRNAs are expressed by women who will subsequently develop term preeclampsia [34]. The appropriate identification of fetuses at risk in women with preeclampsia may allow the establishment of objective criteria on the timing of delivery (term or preterm) according to both fetal and maternal interests [35,36]. A balance between planned deliveries and expectant management is a priority for preeclampsia-/eclampsia-complicated gestations. These approaches might be aided in the future by the assessment and measurement of the products released by the trophoblast, and might help to predict adverse outcomes (maternal and fetal) in these complicated pregnancies.

Technological advances in the study of exosomes and microRNAs can open a new perspective for the early diagnosis and management of preeclampsia and other hypertensive disorders of pregnancy. As Nobel prize winner Ivan Pavlov said each advance in methodology can improve the future of medicine. It is expected that these cross-talks between the trophoblast and the mother could produce new technical diagnostic and clinical approaches that may improve maternal-fetal outcomes of preeclampsia-complicated gestations.

Preeclampsia and later fetal and maternal endocrine and metabolic risks

The long-term health of individuals prenatally exposed to preeclampsia/eclampsia may be seriously impaired [37-39]. In this sense, there is a need to find new trophoblastic products which can be linked to the long-term health of children and adults exposed to preeclampsia. For now, it is uncertain whether trophoblastic EVs, microparticles, exosomes and microRNA are capable of producing permanent maternal metabolic and endocrine alterations that favor the later development of the metabolic syndrome and cardiovascular risk [40-43]. Although preeclampsia and eclampsia are associated with an increased risk of development of hypertension, excessive body weight, high circulating lipids and glucose alterations in later life[44], the relationship between trophoblast-derived products and these derangements remains to be determined.

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