Microvesicles in Thrombosis and Inflammation

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Extracellular membrane vesicles (EVs) are secreted by different types of cells, including blood cells, endothelial, trophoblast, cardiac and tumor cells [1]. EVs are present in the blood circulation and other biological fluids under normal physiologic conditions, and their levels increase in a wide range of disease states. Since EVs contain proteins, growth and apoptotic factors, DNA fragments, microRNAs as well as messenger RNAs (mRNAs), they may function as regulators in cell-cell communication and as mediators of paracrine signaling during multiple biological processes [1]. Depending on their size, mechanism of release and protein composition, EVs can be divided into three subpopulations: exosomes, microparticles (MPs), and apoptotic bodies [2,3].

Microvesicles play a role in physiological and pathologic states

- **Exosomes** are 30–100 nm in diameter and derive from endosomal compartments [4]
- **Microparticles** are larger (ranging from 100 nm to 1 μm) and are released from the cell surface plasma membrane via a process called vesiculation [5]. MPs are shed from the cell membrane upon activation or apoptosis and induce cell signaling that may lead to a variety of processes including invasion, migration, proliferation, angiogenesis or apoptosis [6]. Thus, MPs are involved in thrombosis, inflammation and vascular dysfunction [1]. MPs contain characteristic proteins that are enriched in lipid rafts and are exposed on their surface [7]. Like exosomes, MPs contain typical marker proteins similar to the secreting cells. In addition, emerging evidence suggests that MPs are not simply a consequence of a disease but rather a factor contributing to its pathological processes. Thus, MPs serve as both markers and mediators of vascular complications [8] and may play a significant role in the maternal-placental cross-talk [9]
- **Apoptotic bodies** are released from blebs of apoptotic cells and measure 1–5 μm in diameter [10].

This review focuses on MPs and exosomes that are collectively termed microvesicles (MVs). Upon release, MVs can interact with target cells via a receptor-mediated mechanism [11] or they can directly fuse with the plasma membrane of target cells and release their content into the recipient cell [3]. Alternatively, MVs can be internalized via endocytosis [12]. After internalization, MVs can fuse with endosomes to release the content into the cytosol of target cells; they can be transferred to lysosomes and are degraded.

MICROVESICLES AND THROMBOSIS

Tissue factor (TF), the main activator of the coagulation cascade, is expressed on non-vascular cells, activated cells within the vessel wall (such as leukocytes and endothelial cells), and circulating MVs [13]. TF-bearing MVs arise from raft-rich regions of the plasma membrane enriched with TF, P-selectin glycoprotein ligand-1 and phosphatidylserine [14], and play a central role in the initiation of the coagulation cascade. These MVs have been shown to accumulate during cell injury-induced clot formation and promote thrombotic events [15]. In addition, TF-bearing MVs participate in platelet thrombus formation by binding to P-selectin on the platelet surface [7]. Despite circulation of TF-bearing MVs in healthy people, TF activity remains undetectable and increases upon recruitment to a site of vascular injury. However, in pathological states, MVs bearing active TF confer a predisposition to thromboembolic events [16]. The pro-coagulant properties of MVs may also be attributed to the hemostatic balance between pro- and anti-coagulant mechanisms. It was found that the MV hemostatic ratio between TF and its inhibitor, TF pathway inhibitor (TFPI), was < 1 in healthy controls but significantly increased in patients with any of the following: cardiovascular complications: coronary artery disease (CAD), diabetic CAD (DCAD), diabetic foot [17], solid tumors [18], and hematologic malignancy [19]. Additionally, some MVs express high levels of negatively charged phospholipids such as phosphatidylserine, which provides a catalytic site for coagulation complexes (TF/VIIa, prothrombinase and tenase), thereby indirectly enhancing coagulation activation [20].

High levels of circulating MVs, specifically annexin V-bearing MV, endothelial and platelet MVs, have been associated with an increased risk of venous thromboembolism (VTE) in patients with factor V Leiden and prothrombin G20210A mutation, and in carriers of natural anticoagulant

deficiencies (antithrombin, protein C and protein S defect), suggesting a possible contribution of MVs to the hypercoagulability of genetic thrombophilia. Moreover, an increase in platelet and endothelial MVs was also found in patients with antiphospholipid syndrome (APS) and antiphospholipid antibodies (aPL), a syndrome associated with thrombosis and recurrent pregnancy loss [21].

Microvesicles contribute to hypercoagulation, thrombosis, inflammation and endothelial dysfunction

MICROVESICLES AND INFLAMMATION

Sepsis is a systemic inflammatory response to infection. It is characterized by activation of the coagulation system, inhibition of anticoagulant mechanisms, and fibrinolysis, resulting in disseminated intravascular coagulation with microvascular thrombosis. The up-regulation of inflammatory responses leads to vascular hyporeactivity and enhanced cell apoptosis, which contribute to multiple organ dysfunction and septic shock. Cell activation and apoptosis bring about increased shedding of procoagulant MVs that can activate the coagulation cascade. MVs adhere to leukocytes and increase phagocytic activity. They deliver arachidonic acid (AA) to endothelial cells (ECs) and augment metabolism of thromboxane A2 (TXA2) and expression of COX-2 (mediating prostaglandin formation and enhanced inflammation) [22]. Our previous study demonstrated that MVs, shedding from monocyte cells under inflammatory conditions such as exposure to lipopolysaccharide, bear active TF on their surface, leading to elevated EC thrombogenicity as a result of an increase in TF and decrease in anticoagulant TFPI and thrombomodulin. In addition, inflammatory monocyte MVs can induce EC apoptosis. Exposure of ECs to monocyte MVs results in cell injury and shedding of endothelial MVs from the cell surface; this accelerates the inflammatory response, aggravating coagulation imbalance and impairing angiogenesis [23]. MVs also amplify systemic inflammation via thrombin-dependent activation of the complement [24].

MICROVESICLES, PREGNANCY, THROMBOSIS AND INFLAMMATION

Studies measuring the number of circulating MVs and their cell origin in normal and complicated pregnancy are inconsistent and demonstrate high variation in their results mainly because of the lack of standardization and sensitive tools for MV analysis [25]. The levels of total MVs, platelet-, endothelial- and leukocyte-derived MVs, and tissue factor-bearing MVs in a normal healthy pregnancy were found to be higher in the first trimester as compared to the non-pregnant state, increasing gradually during pregnancy, with the highest values reached in the third trimester [26]. Compared to normal pregnancies, in women with gestational vascular complications (GVC) such as hypertension and preeclampsia, further increases were found in the levels of endothelial MVs, which may indicate a vascular injury [27], and in the levels of monocyte and leukocyte MVs, which may indicate an inflammatory response [28]. Moreover, in non-pregnant women with a history of recurrent pregnancy loss, a significant increase in total annexin V, TF and endothelial MVs was demonstrated compared to parous controls [29]. MVs obtained from healthy pregnant women displayed a higher procoagulant activity compared to those of non-pregnant females [26,30] and an increase in the MV TF/TFPI ratio. The MV procoagulant activity as well as the TF/TFPI ratio appeared to be further elevated in MVs of women with GVC. The presence of increased levels of endothelial-, TF- and phosphatidylserine-expressing MVs at least 3 months after the pregnancy loss suggests continued chronic endothelial damage [29].

Microvesicles obtained from women with gestational vascular complications expressed higher levels of pro-inflammatory cytokines compared to healthy pregnant women

PLACENTAL MICROVESICLES

Circulating MVs of pregnant women include MVs of placental syncytiotrophoblast origin that can be detected in maternal circulation from the second trimester and their number increases during the third trimester [31]. Exosomes released by trophoblasts carry molecules involved in placental physiology and play a key role in cell-cell communication within the placental microenvironment and in maternal-fetal cross-talk [32]. We found that levels of placental trophoblast MVs were similar in all pregnancy groups [28]. However, other publications reported excess shedding of syncytiotrophoblast MVs in early-onset preeclampsia, but not in woman with normotensive intrauterine growth restriction [31,33]. Higher amounts of circulating syncytiotrophoblast MVs in maternal blood might lead to endothelial dysfunction, monocyte stimulation, and an excessive maternal inflammatory reaction [34,35]. Syncytiotrophoblast MVs bearing TF, and other coagulation factors, may reflect the delicate hemostatic balance between maternal and placental cells [36]. Whereas the relative contribution of syncytiotrophoblast MVs bearing TF was substantial in healthy pregnant women, syncytiotrophoblast MVs are among the most increased MVs during preeclampsia and may play an important role in the pathogenesis of this syndrome [37].

A significant increase in the maternal source of TF-bearing MVs in pregnant women with GVC potentially reflects the systemic nature of such pathologies [36]. Syncytiotrophoblast MVs were found to trigger thrombin generation in normal plasma in a TF-dependent manner, which was more pronounced in syncytiotrophoblast MVs shed from preeclamptic placenta, indicating that TF activity is expressed by these MVs [30]. The MV content (proteins, miRNA) and their effects on endothelial and trophoblast cell function vary according to the physiological/

pathological state of a pregnant woman. There is sustained evidence that MVs of women with GVC reflect the pathophysiological state of the patients.

MICROVESICLES, PREGNANCY AND INFLAMMATION

Preeclampsia alters the production of immunoregulatory cytokines and angiogenic factors, resulting in poor trophoblastic invasion at the first stage of the disease; this affects the systemic maternal inflammatory response in the second stage, which includes release of necrotic and/or apoptotic syncytiotrophoblast bodies into the maternal circulation, inducing maternal vascular endothelial injury.

We found that MVs obtained from women with GVC demonstrated higher levels of inflammatory and angiogenic proteins compared with those of healthy pregnant women [38]. Placental MVs can modulate basal peripheral immune cell activation and responsiveness to lipopolysaccharide during normal pregnancy; in preeclampsia, this effect is exacerbated. Placental syncytiotrophoblast MVs generated in vitro from normal placentas stimulate peripheral blood monocytes, which may indicate the contribution of syncytiotrophoblast MVs to the systemic maternal inflammation. Syncytiotrophoblast MVs derived from preeclamptic placentas were found to upregulate the cell surface expression of intercellular adhesion molecule 1 (ICAM-1, CD54) of peripheral blood monocytes, and stimulate the secretion of pro-inflammatory interleukin (IL)-6 and IL-8 from these cells [34]. Inflammatory priming of peripheral blood mononuclear cells (PBMCs) during pregnancy is established by the first trimester and is associated with early inhibition of interferon-gamma (IFNγ) production. The inflammatory response is enhanced in preeclampsia with loss of the IFNγ suppression [39]. Circulating syncytiotrophoblast membrane MVs (STBMs) bind to monocytes and stimulate the production of inflammatory cytokines [39]. MVs derived from hypoxic trophoblasts induce a more intense and rapid inflammatory response of PBMCs than MVs from normal trophoblasts. This difference might explain the exaggerated systemic inflammatory response as a result of placental hypoxia in preeclampsia [40].

Apoptosis is crucial in mediating immune privilege of the fetus during pregnancy. Exosomes secreted by human placenta carry functional Fas ligand, trail molecules and convey apoptosis in activated immune cells, suggesting exosome-mediated immune privilege of the fetus. MVs of healthy pregnant women were found to reduce apoptosis, increase migration, and induce tube formation of endothelial cells. These processes were suppressed by MVs of women with GVC. In early-stage trophoblasts, MVs of healthy pregnant women decreased apoptosis compared with untreated cells and induced higher migration. This effect was mediated through the extracellular signal-regulated kinase pathway. Conversely, MVs of women with GVC increased term trophoblast apoptosis compared to cells exposed to MVs of healthy pregnant women and inhibited early-stage trophoblast migration. Trophoblast debris obtained from culturing placental explants of normal placentas shows markers of apoptosis and is phagocytosed by macrophages or endothelial cells, producing a tolerant phenotype in the phagocyte. When normal placental explants are cultured with antiphospholipid antibodies (a maternal risk factor for preeclampsia), or IL-6 (which increases in the serum of preeclamptic women), the death process in the syncytiotrophoblasts results in more necrotic debris which in turn leads to activation of endothelial cells.

In summary, MVs play a role in physiological and pathologic states. While regulating physiological processes such as coagulation, angiogenesis and endothelial function in pathologic states, MVs contribute to hyper-coagulation, thrombosis, inflammation and endothelial dysfunction.

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