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Review article

Characteristics of erythrocyte-derived microvesicles and its relation with atherosclerosis

Kai-Yin Li [b], Lei Zheng [a], Qian Wang [a], Yan-Wei Hu [a, *]

[a] Laboratory Medicine Center, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China
[b] Institute of Cardiovascular Disease, Key Laboratory for Arteriosclerology of Hunan Province, University of South China, Hengyang, Hunan, China

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ABSTRACT

Microvesicles are formed under many circumstances, especially in atheromatous plaques. Erythrocyte-derived microvesicles (ErMVs) have been proved to promote atherosclerosis by promoting hypercoagulation, mediating inflammation and inducing cell adhesion. Several clinical studies have reported potential roles of ErMVs in cardiovascular disease diagnosis, but the current understanding of ErMVs remains insufficient. In this paper, we will review current research on the formation and degradation of ErMVs and the possible effects of ErMVs in atherosclerosis, discuss potential clinical applications in cardiovascular disease, and hope to raise awareness of the relation with atherosclerosis.

1. Introduction

1.1. Definition of atherosclerosis

Atherosclerosis, a common vascular disease associated with severe health problems, continues to be a major cause of morbidity and mortality, especially in Western countries. Pathologically, the onset of atherosclerosis is characterized by the accumulation of lipid-laden cells beneath the endothelium and formation of atheroma, or fatty deposits, in the inner lining (intima) of the arteries [1]. Without intervention, the atheromatous process prevents blood flow due to blockage of the distal vessels, which can result in acute ischemic events that reduce both survival and quality of life. Many hypotheses have been proposed to explain the onset and progression of atherosclerosis [2–6]. Thrombin generation has been reported to play an important role in both the early stages of atheroma formation and plaque progression and destabilization [7]. Atherosclerosis is considered a result of inflammatory and immune responses to accumulation of lipoproteins and lipids in the arterial walls. Altered adhesion is also an important pathogenic process in atherosclerosis [8]. Microvesicles from monocytes, smooth muscle cells, and platelets have been reported to play important roles in the process of atherosclerosis. Erythrocytes, which are the most abundant blood cell type, also contribute to this process.

1.2. Erythrocyte-derived microvesicles (ErMVs)

1.2.1. Formation and degradation

Extracellular vesicles includes “exosomes” (diameter <200 nm formed via an endocytic pathway) and “microvesicles (MVs)” (≤1 μm in diameter formed via budding of the plasma membrane) [9]. Microvesicles derive from various types of cells, including platelets (PLTs), monocytes, ECs, erythrocytes, and granulocytes, among others [10]. When erythrocytes encounter specific stimuli [11], such as shear stress, complement attack, agonist (or pro-apoptotic) stimulation, or damage, phosphatidylserine (PS), which is located almost exclusively in the inner layer of the cell membrane under normal conditions [12], moves to the outer membrane, indicating that membrane anchorage to the intracellular cytoskeleton is disrupted, thereby promoting the formation of MVs (Fig. 1) [13]. This process requires an increase in the amount of intracellular calcium, changes in membrane lipid symmetry, and cytoskeleton reorganization, which may be caspase-related [14–16]. Besides,

* Corresponding author. Laboratory Medicine Center, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong 510515, China.
E-mail address: ywhu0618@163.com (Y.-W. Hu).

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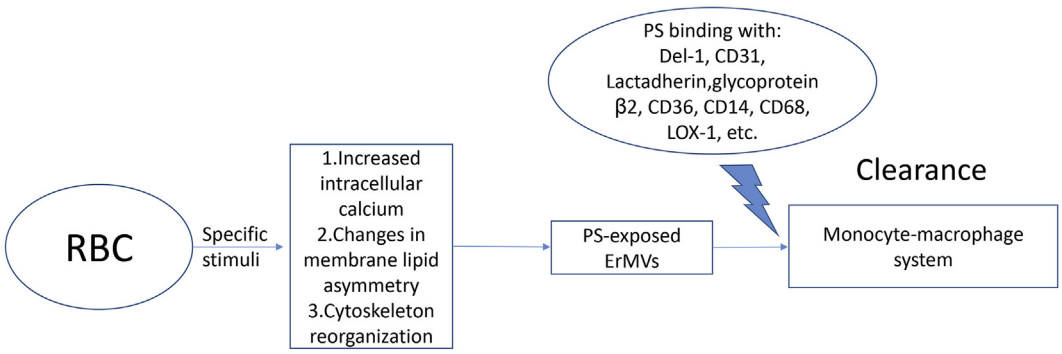

Fig. 1. Erythrocyte-derived microvesicles formation and degradation. When erythrocytes encounter specific stimuli, such as shear stress, complement attack, agonist (or pro-apoptotic) stimulation, or damage, they are prone to form microvesicles (MVs) as an escape from apoptosis. This process requires increased intracellular calcium, changes in membrane lipid asymmetry, and cytoskeleton reorganization. The clearance of circulating erythrocyte-derived microvesicles (ErMVs) occur mainly through interactions with specific receptors and related molecules, in the monocyte-macrophage system, including liver Kupffer cells, lungs, and bone marrow.

hemoglobin (Hb) continually binds and releases molecular oxygen, while abnormal Hb-O_2 electron transfer generates superoxides, including methemoglobin (metHb) and subsequent hemichromes, as well as other related reactive species that may then oxidize and/or cross-link Hb, cytoskeletal proteins, membrane proteins, and membrane lipids [11]. This auto-oxidation of Hb occurs in 0.5%–3% of total Hb every day. Studies have demonstrated that oxidation of the cytosolic face of band-3, a transmembrane chloride/bicarbonate ion exchanger that also binds to the underlying cytoskeleton, leads to tyrosine phosphorylation of band-3, resulting in the dissociation of band-3 from the underlying cytoskeleton and vesiculation. ErMVs are generated throughout the lifecycle of erythrocytes. It is reported that over 20% of intracellular Hb will be lost through various mechanisms, including shedding into MVs, and over 30% of the volume of an erythrocyte will be lost as it ages [11,17]. Clearance of ErMVs is normally related to PS-specific receptors expressed by the monocyte-macrophage system in liver Kupffer cells, bone marrow, and lungs [18]. PS directly induces the degradation process and through the receptors that include developmental endothelial locus 1 on ECs and CD31 and lactadherin (milk fat globule-epidermal factor 8) on the macrophage membrane [19,20]. Besides, other receptors, such as glycoprotein â$_2$, CD36, CD14, CD68, and lectin-like oxidized low-density lipoprotein receptor-1 might also be related to the uptake of MVs via PS-specific binding [15,21,22]. Receptor-ligand binding plays an important role in MV uptake. However, a study showed that a portion of MVs may not express PS on the surface *in vivo* [20], so clearance of this portion of MVs remains unclear and may be accounted for as just a low rate of PS-induced processes or even through a different mechanism altogether.

1.2.2. Features

Loss of asymmetry occurs in MVs from different resources, some of which are featured by membrane expression of PS. MVs carry source cells-derived antigens and lipids, and contain biological macromolecules, including proteins, microRNA, and so on [23,24], which indicates a possible mechanism to transfer protein and RNA to other cells to modulate angiogenesis and reprograming of hematopoietic stem cells [25]. A study has shown altered membrane composition in ErMVs, as compared to normal erythrocytes. Membrane-bound CD47 and Crry share the same density in erythrocytes, but there is an obvious reduction in DNA-amplified fingerprints and CD59a [17]. Besides, ErMVs carry more Fas-related molecules, including Fas/CD95, Fas-associated death domain (FADD), and caspases 3 and 8 as blood unit storage with prolonged time [11,26].

2. ErMVs and atherosclerosis

ErMVs can influence atherosclerosis directly and indirectly by promoting a hypercoagulation state through multiple mechanisms, including both enhancement of pro-coagulation activities and inhibition of anti-coagulation mechanisms, and are possibly important mediators between these two processes. Atherosclerosis is considered a state of chronic inflammation of the vessel wall, in a pathogenesis partially regulated by ErMVs through inflammatory cells, inflammatory factors and oxidative stress. As for adhesion, ErMVs might influence this important process through PS exposure and oxidative stress. Considering this, the authors think ErMVs may provide a new orientation for fundamental and clinical research in atherosclerosis. Currently, most studies have been based on isolated erythrocyte units. Therefore, the authors will conclude what has been found between ErMVs and atherosclerosis, and hope to raise awareness. In the following sections, we will review current research of possible effects of ErMVs in atherosclerosis through the above-described mechanisms (Fig. 2).

2.1. ErMVs and coagulation

Elevated levels of circulating ErMVs are associated with increased thrombin generation *in vivo* [13]. Hence, ErMVs can be recognized as markers of ongoing coronary thrombosis [27]. MVs with zymoplastic potential exist mainly in atheromatous plaques, most of which is derived from monocytes (29 ± 5%), erythrocytes (27 ± 4%), and lymphocytes (15 ± 3%) [27], and induces the coagulation process, through both the intrinsic and extrinsic pathways of thrombin. Van Der Meijden et al. [28] reported that deficiency of factor VII has no effect on ErMV-induced thrombin production *ex vivo*, while a lack of factor XII results in the absence of thrombin production, which suggests that ErMVs promote thrombin production via factor XII_a, which activates factor XI and initiates intrinsic pathways. Besides, a feature of ErMVs is the loss of membrane asymmetry, which can result in exposure of PS. The latter has been reported to activate the extrinsic pathway via tissue factor (TF) *in vivo* [29,30], which binds to factor VII_a, and then induced production of factors X_a and IX_a. On the one hand, factor IX_a can activate factor X through interactions with factor $VIII_a$, while factor IX_a can also activate factor VII, which is associated with intrinsic and extrinsic pathways, and eventually results in the production of fibrin from fibrinogen. In addition, PS can also bind with the CD36 scavenger receptor on the platelet membrane, which induces adenosine diphosphate-related platelet activation *in vivo* [31]. Another experiment found that purified ErMVs in atheromatous plaque displayed

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