Pathophysiology 23 (2016) 265–274

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Review

From trash to treasure: The untapped potential of endothelial microparticles in neurovascular diseases

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ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 4 August 2016
Accepted 12 August 2016

Keywords:
Alzheimer's
Multiple sclerosis
Endothelial
Platelet

ABSTRACT

Discovered in 1947, microparticles (MP) represent a group of sub-micron cell-derived particles isolated by high speed centrifugation. Once regarded as cellular 'trash', in the past decade MP have gained tremendous attention in both basic sciences and medical research both as biomarkers and mediators of infection, injury and response to therapy. Because MP bear cell surface markers derived from parent cells, accumulate in extracellular fluids (plasma, serum, milk, urine, cerebrospinal fluid) MP based tests are being developed commercially as important components in 'liquid biopsy' approaches, providing valuable readouts in cardiovascular disease and cancer, as well as stroke, Alzheimer's disease and Multiple Sclerosis. Importantly, MP have been reported as mobile transport vectors in the intercellular transfer of mRNAs, microRNAs, lipids and proteins. Here we discuss MP structure, properties and functions with particular relevance to neurological and neurovascular diseases.

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http://dx.doi.org/10.1016/j.pathophys.2016.08.004
0928-4680/

1. Introduction

In 1947, Erwin Chargaff and Randolph West reported that supernatants obtained from high-speed centrifugation of plasma showed a significantly longer clotting time [22]. Interestingly, the restoration of the pellet from this procedure back to the supernatant restored clotting and shortened the clotting time. Twenty years later, in 1967, Peter Wolf at the University of Birmingham used electron microscopy to further characterize the pro-coagulant component of plasma that was removed by high speed centrifugation [121] as particles in these pellets which were apparently derived from activated platelets. He described these sub-micron particles as platelet '*dust*'; he further noted that this dust also contained phospholipids. Although initially these particles were dismissed as cellular '*trash*', in the past decade these so-called 'microparticles' (MP) have gained much more attention in both basic sciences and medical research as both markers and mediators of infection, injury and response to therapy.

The advancement of scientific knowledge on MPs as well as improved technology has now permitted a much more extensive analysis of these submicron particles, which are now widely described as MPs, or sometimes 'microvesicles'. These structures were defined by the International Society on Thrombosis and Haemostasis Vascular Biology Subcommittee in 2005 as "0.1–1.0 μm cell-derived vesicular structures that lack a nucleus or synthetic capability, but often contain membrane cytoskeleton. MPs are also distinguished from other extracellular vesicles such as exosomes, which are smaller (40–100 nm in size [64]) and more homogenous population of vesicles of endosomic origin, and apoptotic bodies, which are larger (1–4 μm) and are formed during the late stages of apoptosis.

These diverse particle groupings differ not only in sizes but also in isolation methods. A consensus isolation method for MP involves centrifugation at 15,000 to 20,000*g* for 45–90 min, which is different from exosome isolation (refer to Table 1). Exosomes are isolated by centrifugation at much higher speeds, typically 100,000 × *g* [107]. Not only are exosomes and MPs segregated by their method of preparation; they are also biochemically and morphologically distinct with exosomes having no, or very low, annexin V binding capacity and no ability to bind pro-thrombin or factor X [28]. Therefore MPs appear to represent the major pro-coagulant species described by Chargaff and West as well as Wolf.

MPs have been found to frequently retain several 'parent' cell surface markers as well as phosphatidyl serine (PS), which binds annexin (V) on the outer leaflets of their plasma membranes and have a limited cytoplasm, derived from the originating cell. Most importantly, many groups, including ours, have shown that MPs are not only released by activated platelets but from most, if not all, cell types, where they can accumulate in the extracellular environment *e.g.* plasma, serum [19]. MPs are cleared from several sources through diverse mechanisms. MP are found not only in the blood spaces but also in the urine [102], cerebrospinal fluid [56] and even milk [88]. Many diverse disease states, including cancer [39], kidney diseases (especially chronic renal failure), [2], Alzheimer's disease [123] and Multiple Sclerosis [49], have reported elevations in MP levels which are linked with disease onset, severity and effectiveness of therapy. Perhaps most importantly because MPs can be viewed as a 'sampling' of the cell surface and cytoplasm of the parent cells under different states (quiescent, activated, apoptotic), the quantity and/or compositions of MPs in bodily fluids appears to serve as a highly convenient and relatively noninvasive biomarkers as well as indices of pathophysiology in many clinical conditions.

Furthermore, recent advances have now also reported the importance of MP as vectors in the transport and even intercellular transfer of several forms of cytoplasmic information including microRNA [20], bioactive lipids [44], and proteins [70], strongly supporting their roles as under-recognized mobile signal modules in addition to their participation as effectors of pathophysiology. This review considers and summarizes our current understanding on the properties and composition of MPs, their possible functions, relevance to and implication in various diseases.

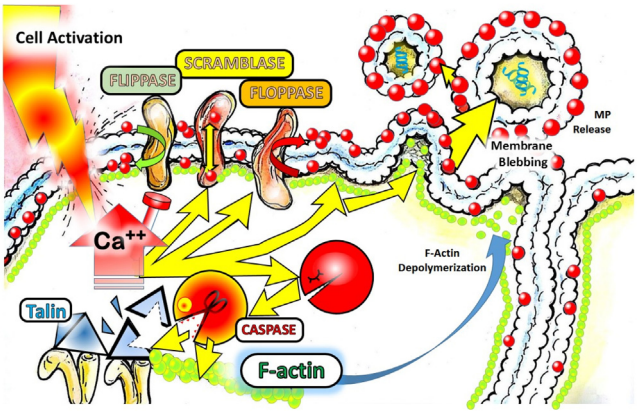

Fig. 1. Generation of MP from activated cells.

2. Characterization of MPs

MPs are small, membrane-enclosed parcels of cytoplasm which represent 'fragments' of cells that form by a process of direct budding off of the plasma membrane. MPs are defined as particles between 100 and 1000 nm in diameter, which typically exhibit phosphatidylserine (PS) on their outer leaflet of the plasma membrane. MP size distributions can be identified by electron microscopy; increasingly MPs are being typed and identified using fluorescence activated cell sorting (FACS). The standard procedure has been to affinity stain MP PS using annexin V, which binds to PS that is exposed on the external surface. This is a convenient method, since MPs are frequently released from pro-apoptotic and activated cells. The basis of this detection method underlies the fact that 'normal' (quiescent) cells maintain plasma membrane asymmetry, keeping PS exclusively in the inner leaflet [86]. This asymmetry is established by the ATP-dependent enzyme, '*flippase*' [9]. Two other important enzymes, '*floppase*', translocates phospholipids from the inner to outer membrane leaflets, while '*scramblase*', causes random bidirectional movements of phospholipids; both are normally inactive in 'healthy' cells [128] [103].

A common event in MP generation relates to increased transmembrane ion fluxes (Fig. 1). During cell activation, intracellular calcium levels rise, inhibiting flippase and activating both floppase and scramblase, which lead to a loss of heterogeneous membrane asymmetry and the presentation of PS on the outer leaflet of the cell membrane. Elevated cell calcium also activates *calpain*, which is also normally inactive in the cytosol until rising cytosolic calcium levels promote its translocation to the inner leaflet of the membrane, where calpain is active. Activated calpain promotes cytoskeletal reorganization by cleaving talin and actin filaments on the plasma membrane, which facilitate the release of MPs [23]. Calpain is therefore considered to be an essential protease in the generation of MPs during cell activation. Basal MP release from resting platelets however, depends on αIβ3 integrin signaling, again with cytoskeletal destabilization and turnover, but without the increased intracellular calcium levels or calpain activation [21].

Conversely, the central proteases mediating the release of MPs from *apoptotic* cells are *caspases*. Caspase-3 targets Rho kinase (ROCK), and cleaves its inhibitory domain. This ROCK I become constitutively active, which then stimulates the contractility of the inner cell membrane both directly, *via* phosphorylation of myosin