REVIEW ARTICLE

Microparticles Novel Mechanisms of Intracellular Communication: Implication in Health and Disease

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Abstract

BACKGROUND: The prevailing view that eukaryotic cells are restrained from intercellular exchange of genetic information has been challenged by recent reports on nanotubes, exosomes, apoptotic bodies, and nucleic acid—binding peptides that provide novel pathways for cell—cell communication, with implications in health and disease.

CONTENT: Microparticles (MPs) are a heterogeneous population of small plasma membrane structures that serve as important signaling structures between cells. MPs are composed of a phospholipid bilayer that exposes transmembrane proteins and receptors and encloses cytosolic components such as enzymes, transcription factors, and mRNA derived from their parent cells. Growing evidence suggests that MPs regulate inflammation, stimulate coagulation, affect vascular functions and apoptosis, and can also play a role in cell proliferation or differentiation. MPs circulate in the bloodstream, can be detected in the peripheral blood, and may originate from different vascular cell types (eg, platelets, monocytes, endothelial cells, red blood cells, and granulocytes).

SUMMARY: Cells of various types release small membrane vesicles called MP on their activation, as well as during the process of apoptosis. The properties and roles of MP generated in different contexts are diverse and are determined by their parent cell and the pathway of their generation, which affects their content. MP are involved in multiple cellular functions, including immunomodulation, inflammation, coagulation, and intercellular communication. MPs are able to deliver molecular signals in the form of lipids, proteins, nucleic acids, or functional trans-membrane proteins from the parent cell to distantly located targets. From a clinical point of view, MP may serve as biomarkers for disease status and may be found useful for developing novel therapeutic strategies.

KEYWORDS: Microparticles, Microvesicle, Membrane Remodeling, Intercellular communication.

Indones Biomed J 2011; 3(1): 18–36

Introduction

Cells communicate and exchange information by different mechanisms. They may communicate by (i) secreted growth factors, cytokines, chemokines and small molecular mediators (e.g., nucleotides, nitric oxide ions, bioactive lipids), (ii) cell-to-cell adhesion contacts that are mediated by sets of specialized adhesion molecules and (iii) exchanging information by means of tunneling nanotubules (1-6). However, attention is now being focused on cell-to-cell communication that involves circular membrane fragments called microparticle (MP) (7-12), a mechanism that for many years has been largely overlooked.

DOI: 10.18585/inabj.v3i1.131
Indones Biomed J. 2011; 3(1): 18-36

It is well established that virtually all eukaryotic cells possess the fundamental capacity to release small vesicles, generally referred to as MPs. Existence of MPs, which allow a selective and concentrated release of the cellular content into the surrounding milieu, was first noticed by Wolf in 1967 as formation of a procoagulant "dust" around activated blood platelets (13). Although MPs are present in peripheral blood of healthy individuals, with platelet MPs being the most abundant and representing 70% to 90% of all circulating MPs (14,15) marked elevations occur in many disease states. These conditions include autoimmune disorders, atherosclerosis, malignancies, and infection among others (14,16-18).

Shedding of membrane-derived MP is a physiological phenomenon that accompanies cell activation and growth (7-12,15,19). Interestingly, rapidly growing cell lines tend to secrete more MP than slowly growing ones. Generally, the number of MP shed from cells increases upon (i) cell activation, (ii) hypoxia or irradiation, (iii) oxidative injury, (iv) exposure to proteins from an activated complement cascade and (v) exposure to shearing stress (7-12,15,19). MP shedding depends on an increase in cytosolic Ca^{2+} and degradation of the membrane skeleton.

Although a precise definition of MPs remains elusive, they are commonly described as a heterogeneous population of spherical structures with a diameter of 100 to 1000 nm, which are released by budding of the plasma membrane (ectocytosis) as phospholipid vesicles known to express antigens specific of their parental cells. This characterization allows differentiation from exosomes, referring to preformed vesicles with are diameter of less than 100 nm that are stored intracellularly in multivesicular compartments and are secreted when these endosomal compartments fuse with the cell plasma membrane (20,21). Continuing efforts in deciphering the signature of circulating MPs could also lead to the development of new diagnostic strategies, with MPs emerging as unique potential sources of disease-related and possibly predictive biomarkers (22).

Microparticle (MP)

In multicellular organisms, homeostasis results from a subtle balance between cell proliferation and degenerescence. Cells differentiate, expand, fulfill particular functions, then undergo programmed death and are finally cleared by phagocytosis. At each stage of its life, the cell is subjected to a variety of stimulations leading to the release of submicron fragments from the plasma membrane, usually termed microvesicles or microparticles (MPs). MPs hijack membrane constituents and cytoplasmic content and survive the cell (10).

MPs are fragments shed almost spontaneously from the plasma membrane blebs of virtually all cell types when submitted to a number of stress conditions, including apoptosis. MP release is an integral part of the membrane-remodeling process in which the asymmetric distribution of constitutive phospholipids (PL) between the two leaflets is lost. After having long been considered 'cell dust', MPs have more recently been shown to reflect *in vitro* cell stimulation, and testify to cellular activation and/or tissue degeneration occurring in vivo under a variety of pathophysiologic circumstances. Besides their marker characteristics, MPs have been identified as true vectors in the transcellular exchange of biologic information (23).

On one hand, deleterious MP stemming from activated cells can elicit an adverse response from other cells, themselves undergoing membrane vesiculation, leading to pathogenic amplification. On the other hand, since they are thought to reflect a balance between cell stimulation, proliferation, and death, it is conceivable that they are discerned as sensors for the maintenance of homeostasis in multicellular organisms. Because vesiculation is an integral part of the plasma-membrane remodeling process, with the transverse migration of procoagulant phosphatidylserine from the cytoplasmic to the exoplasmic leaflet as the central event, the majority of released MPs are thought to fulfill a hemostatic function under physiologic conditions. This is particularly true when they originate from platelets, with possible deviation towards thrombosis when produced in excess (23).

It has been postulated that the phenotype of stem cells is reversibly changing during the cell cycle transit until a terminal-differentiating stimulus is encountered at a cycle-susceptible time. In this model the cell cycle status and the microenvironmental exposure to the products of contiguous cells may play key roles in stem cell plasticity (29). The same stem cell may show different phenotypes in different functional states, depending on the cell cycle phase. This dynamic context is regulated by the microenvironment and in particular the MP-mediated transfer of genetic information between cells (30).

Transfer of genetic information from injured cells may explain stem cell functional and phenotypic changes without the need for trans-differentiation into tissue cells. On the contrary, transfer of genetic information from stem cells may redirect altered functions in target cells suggesting that stem cells may repair damaged tissues without directly replacing parenchymal cells (26). MPs

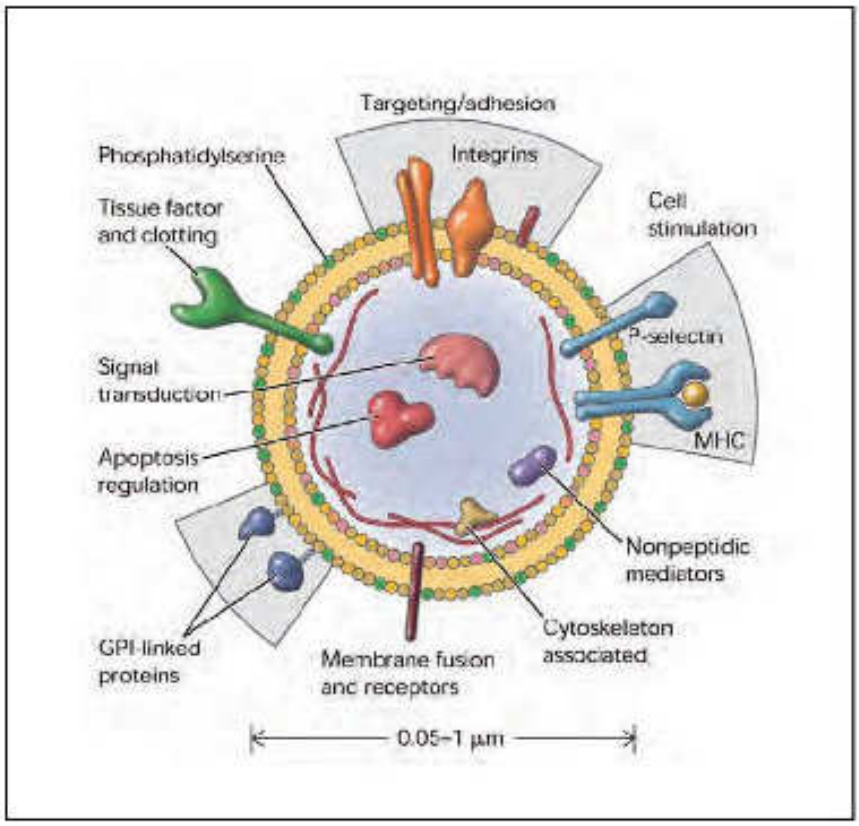

Figure 1. Cellular MP: a disseminated storage pool of bioactive effctors (Adapted with permission from American Physiol Society).

can therefore be considered a disseminated storage pool of bioactive effectors, the nature and proportion of the latter accounting for duality, more particularly evidenced in vascular disease, inflammation, and immunity (10).

Thus, MPs are vesicles that bud off from cells, lack a nucleus, contain a membrane skeleton and are defined by their size and expression on their surface of antigens specific of parental cells (27-29). These phospholipid vesicles are less than 1 μm of diameter. To reliably define MPs, the terms exosomes and ectosomes need to be introduced. Exosomes originate from multivesicular bodies and exocytosis of endocytic bodies, and ectosomes directly originate from the membrane surface (15, 16). In this review, we will mainly use the commonly used term "microparticles", keeping in mind their definition as ectosomes (30,31).

MPs display a broad spectrum of bioactive substances and receptors on their surface and harbor a concentrated set of cytokines, signaling proteins, mRNA, and microRNA. Recent studies provided evidence for the concept of MP as veritable vectors for the intercellular exchange of biological signals and information. Indeed, MP may transfer part of their components and content to selected target cells, thus mediating cell activation, phenotypic modification, and reprogramming of cell function. Because MP readily circulate in the vasculature, they may serve as shuttle modules and signaling transducers not only in their local environment but also at remarkable distance from their site of origin. Altogether, this transcellular delivery system may extend the confines of the limited transcriptome and proteome of recipient cells and establishes a communication network in which specific properties and information among cells can be efficiently shared (22).

Circulating MPs in blood originate from different cells (i.e. red blood cells, granulocytes, monocytes, lymphocytes, platelets and ECs) and their blood levels result from the balance between their rates of release from cells and their clearance from the circulation. Changes in MP levels in circulating blood may be due to some pathological conditions. Platelet – derived MPs (PMPs) are the most abundant, representing about 70–90% of all circulating MPs (17).

Hence, MP could serve as potential diagnostic markers in laboratory medicine and the development of new diagnostic strategies based on the analysis of number and molecular signature of circulating MP can be anticipated in the near future (32).

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Formation of MPs

Generation and shedding of MPs occurs during biological processes of considerable diversity, including not only cellular activation following stimulation with proinflammatory, prothrombotic, or proapoptotic substances, or exposure to high shear stress as present in arteries with a severe stenosis, but also cellular differentiation, senescence, or apoptotic cell breakdown (14,33,34).

The plasma membrane is a well-structured entity characterized by a controlled transverse distribution of lipids and proteins between the two leaflets but also by a lateral organization in domains termed "rafts." Following stimulation, a general redistribution occurs, leading to raft structuration, phosphatidylserine externalization, and MP release (10).

In steady-state, the cell membrane is asymmetric regarding the composition and the distribution of phospholipids in its inner and outer layers: phosphatidylcholine and sphingomyelin are located in the outer layer, while phosphatidylserine (PS) and phosphatidyl-ethanolamine (PE) are present in the inner layer. This asymmetric distribution of phospholipids in the membrane is maintained by a three piece enzyme system: **flippase**, **floppase** and **scramblase**. Flippase is an aminophospholipid translocase that specifically translocates PS and PE from the outside to the inside of the bilayer membrane. Floppase transports phospholipids from the inner to the outer leaflet. Floppase does not specifically act on transport of aminophospholipids and probably works together with flippase. Scramblase, whose role is thought to be the transportation of phospholipids between the two monolayers of the cell membrane, is inactive in steady-state (10,23,35).

The exposure of procoagulant phospholipids and the shedding of MPs are cellular responses that depend on activating conditions. Notably, membrane remodeling and PS externalization is dependent on an increase in cytosolic calcium. Activation of human platelets by a Ca^{2+} ionophore results in the surface exposure of PS. Conversely, the inhibition of Ca^{2+} influx abolishes agonist-induced PS externalization and the procoagulant response in activated platelets (36).

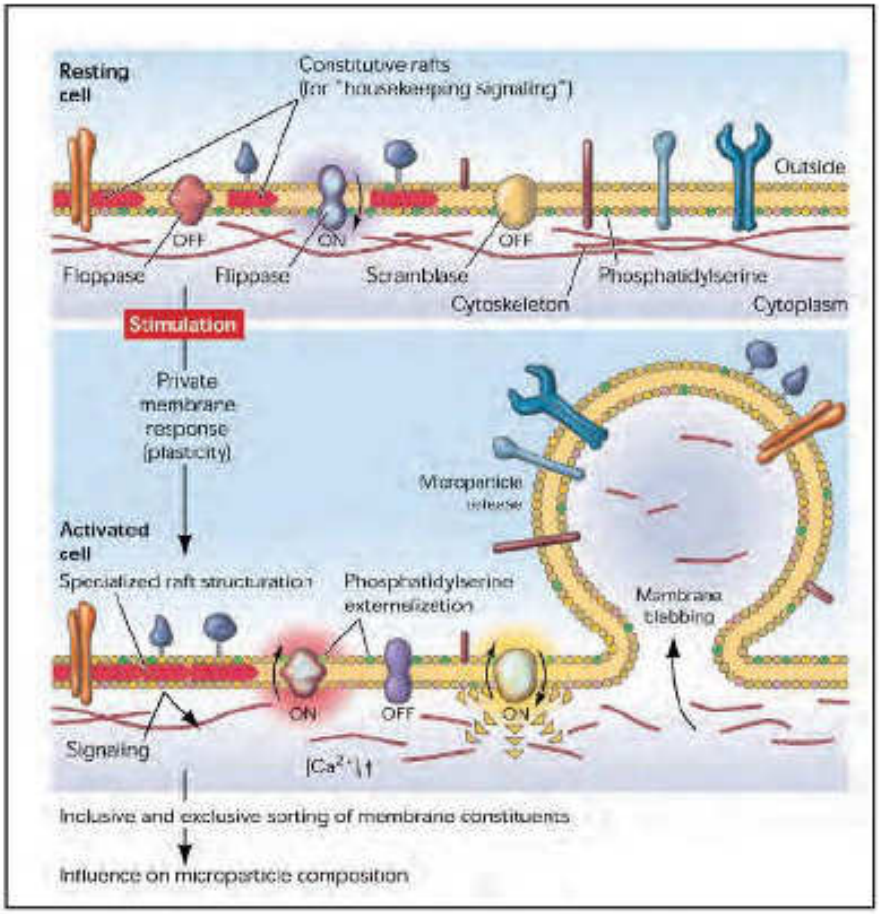

Figure 2. The plasma membrane response to cell stimulation (Adapted with permission from American Physiol Society).

Calcium inactivates flippase and activates floppase and scramblase, inducing the loss of phospholipids asymmetry between the inner and the outer leaflets. Contacts between aminophospholipids and cytoskeleton are then disrupted. In addition, calcium release leads to activation of two enzymes: **calpain** and **gelsolin**. Calpain hydrolyzes actin-binding proteins that decreases association of actin with membranes glycoproteins (38,39), while gelsolin (only in platelets) is involved in the cleavage of the actin capping proteins (40).

As small increases in Ca^{2+} lead to platelet activation, the maintenance of a stable Ca^{2+} is essential to keep platelets in a resting state. The mechanisms limiting platelet activation by counteracting Ca^{2+} leakage from the intracellular stores rely on sarcoplasmic/endoplasmic Ca^{2+} ATPases that pump the calcium ions back to the stores (targeted by the inhibitor thapsigargin) and on a plasma membrane Ca^{2+} ATPase that pumps Ca^{2+} out of the cell (17,41).

When cells undergo activation or apoptosis, PS externalization is one of the earliest observable indicators of the process. Its translocation to the outer leaflet is the initial event that will ultimately lead to the shedding of procoagulant MPs that are therefore regarded as reliable markers of cell stress (42). The dynamic balance of cell stimulation, cell proliferation, and death within the vessels is reflected by the formation and release of MPs that may thus represent a vascular storage pool of bio-effectors (43).

MPs shed from activated, necrotic, or apoptotic cells provide a catalytic phospholipid surface for the assembly of blood coagulation factors, thereby promoting the coagulation cascade and thrombin generation (44). MPs can harbor active tissue factor (TF), the cellular initiator of blood coagulation *in vivo* (45-47). Because PS and TF are known to act synergistically as potent triggers of blood coagulation, it has been suggested that TF-bearing MPs represent the so-called blood-borne TF (48-50). These observations suggest that MPs can be viewed as a major therapeutic target, not only in the inhibition of arterial and venous thrombosis but also in the containment of the systemic inflammatory response and atherosclerosis (51).

The mechanism of MPs clearance from the circulation is not known. Platelets have a life span of about 10 days, contrasting with that of PMPs of which is about 30 minutes in mice (52), or even less than 10 minutes in rabbits (53). These MPs could be cleared from the circulating blood by phospholipases (54), by direct mechanisms such as PS exposure and subsequent phagocytosis, or by indirect mechanisms such as opsonization by proteins such as growth arrest-specific gene 6 product (GAS6), protein S and complement (55).

A wide variety of methods are used to measure, quantify, and phenotype MPs from blood samples or cell culture supernatants. For this reason, critical evaluation and standardization of the different methods used by each laboratory are necessary to reliably compare studies together. Most methods use flow cytometry although the use of classical flow cytometer is subject to caution, as discussed by Bruce and Barbara C. Furie (56). The small size of MPs enhances the difficulties for their detection and quantification.

MPs can be characterized by the detection of the different cell surface antigens (Table 1). These antigens reflect their origin and activation method.

Despite a recent proteomic characterization of tumoral lymphocyte MPs (57), the chemical composition of MPs remains poorly described. MPs contain various proteins inherited from their parental cells and a membranous skeleton. Thereby, their origin can be identified by the presence of cell-specific surface antigens (Table 1). Other components of MPs have been recently described, such as mRNA (58,59), prions (60,61), contractile proteins such as thrombosthenin (62).

Platelet Microparticles

Blood contains MPs derived from different cell types, including mainly platelets, but also red blood cells, granulocytes, monocytes, lymphocytes and endothelial cells (ECs). Overproduction of MPs has been related to various physiological and pathophysiological conditions such as cell adhesion, apoptosis, immune response, vascular function, vascular remodeling and angiogenesis, haemostasis and thrombosis, cardiovascular diseases, cancer, infections, as well as normal and pathological pregnancy.

A surface area unit of PMP has approximately 50- to 100-fold higher procoagulant properties than an identical surface area unit of an activated platelet (63). Thus, the usually accepted role of MPs is to promote coagulation. This is principally due to the presence of TF, the principal initiator of coagulation, exposed on the surface of MPs. Regardless the stimulus, about 25% of the procoagulant activity in blood is associated with MPs derived from activated platelets (64).

A population of PMPs is generated during platelet activation, whereas other PMPs populations are derived from megakaryocytes during megakaryopoiesis (65-67), quiescent circulating platelets or might result from platelet

DOI: 10.18585/inabj.v3i1.131
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Table 1. Markers for cell-derived MPs

Cellular origin of MPs	Marker
Red blood cell	CD235a
Leucocyte	CD45
Granulocyte	CD66b
Monocyte	CD14
Lymphocyte	CD4
	CD8
	CD20
Platelet	CD31
	CD41
	CD41a
	CD42a
	CD42b
	CD61
	CD62P
Endothelial cell	CD31
	CD34
	CD54
	CD62E
	CD51
	CD105
	CD106
	CD144
	CD146

apoptosis (52). PMPs can be produced by various stimuli, including platelet agonists, calcium ionophore, complement-binding proteins, or high shear. After stimulation by the thrombin-receptor agonist peptide (TRAP), MPs from 0.1 μm to 1 μm and exosomes from 40 to 100 nm are released (68). Stimulation of platelets activates intracellular calpain, and calpain inhibitors impair MPs release (39).

PMPs are generated under certain blood flow conditions as well (69). High shear stress in severe atherosclerotic arteries activates platelets, generating PMPs, whereas normal shear stress does not (33). In addition, platelet adhesion to immobilized von Willebrand factor (VWF) under fast flow conditions, engages a mechanism for the generation of MPs. This results in the deposition of procoagulant structures that are not removed even under extreme flow conditions, as encountered in severely stenosed arteries (70). This mechanical release of PMPs is dependent on the interaction of vWf with glycoprotein (GP) Ibα, and the resulting procoagulant PMPs enhance thrombus formation (17).

Apoptosis and vascular cell activation are main contributors to the release of procoagulant MPs, deleterious

partners in atherothrombosis. Elevated levels of circulating platelet, monocyte, or endothelial-derived MPs are associated with most of the cardiovascular risk factors and appear indicative of poor clinical outcome. In addition to being a valuable hallmark of vascular cell damage, MPs are at the crossroad of atherothrombosis processes by exerting direct effects on vascular or blood cells. Under pathological circumstances, circulating MPs would support cellular cross-talk leading to vascular inflammation and tissue remodeling, endothelial dysfunction, leukocyte adhesion, and stimulation. Exposed membrane phosphatidylserine and functional TF are 2 procoagulant entities conveyed by circulating MPs. At sites of vascular injury, P-selectin exposure by activated endothelial cells or platelets leads to the rapid recruitment of MPs bearing the P-selectin glycoprotein ligand-1 and blood-borne TF, thereby triggering coagulation (44).

Endothelial MPs

MPs released into the bloodstream can act as messengers delivering a variety of cargos, such as cell surface receptors, proinflammatory cytokines, signaling molecules, and even mRNA, to distal cells (17,44). They may also contribute to disease by transporting viruses and prions (17,44). In addition, *in vitro* studies have shown that binding of MPs to endothelial cells and monocytes induces the expression of proinflammatory and procoagulant molecules (Figure 3).

Inflammation and coagulation are linked processes in many diseases and MPs may amplify the responses by activating the endothelium. In addition, proinflammatory mediators directly induce tissue factor expression in endothelial cells, and the coagulation protease thrombin directly induces the expression of proinflammatory mediators in endothelial cells. This results in elevated levels of endothelial cell–derived MPs, so-called EMPs, in many disease states. The presence of these EMPs in blood can be used as biomarkers of endothelial cell injury (71,72).

EMP (~100 nm to 1 μm in diameter) result from endothelial plasma membrane blebbing and carry endothelial proteins such as vascular endothelial cadherin, platelet endothelial cell adhesion molecule-1, intercellular cell adhesion molecule (ICAM)-1, endoglin, E-selectin, S-endo or αv integrin (73). Endothelial NO synthase and vascular endothelial growth factor receptor (VEGF-R2) have also been identified on EMP (74), but there is so far no evidence on whether or not MP endothelial nitric oxide synthase is capable of generating nitric oxide; furthermore, endothelial nitric oxide synthase may also be present on platelet or red blood cell–derived MP (75).

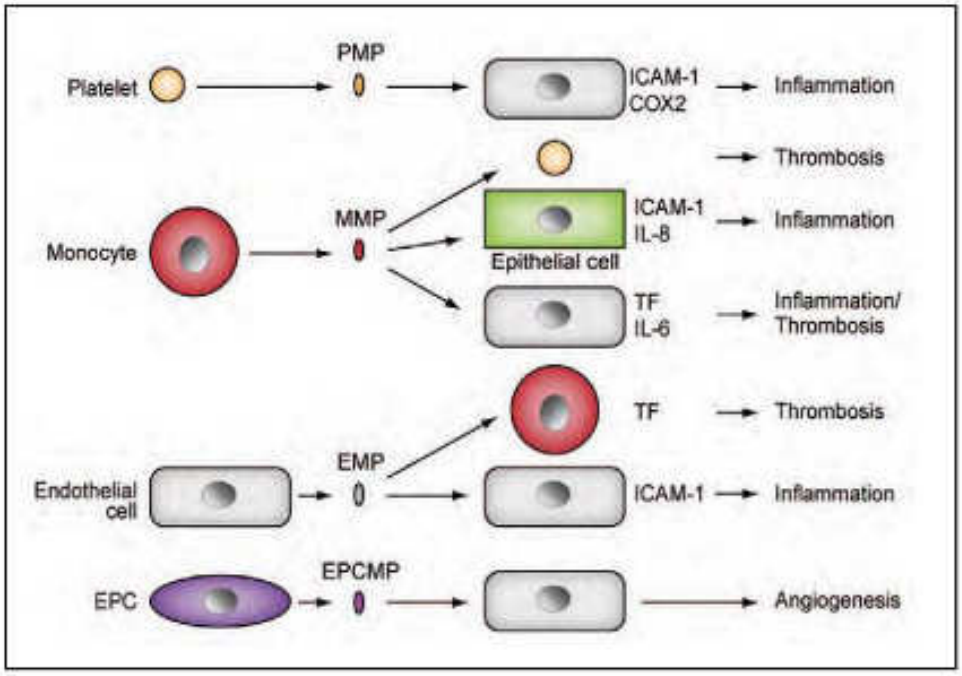

Figure 3. MPs derived from different cell types induce the expression of proinflammatory and procoagulant molecules in endothelial cells, monocytes, and epithelial cells (Adapted with permission from American Heart Asssociation).

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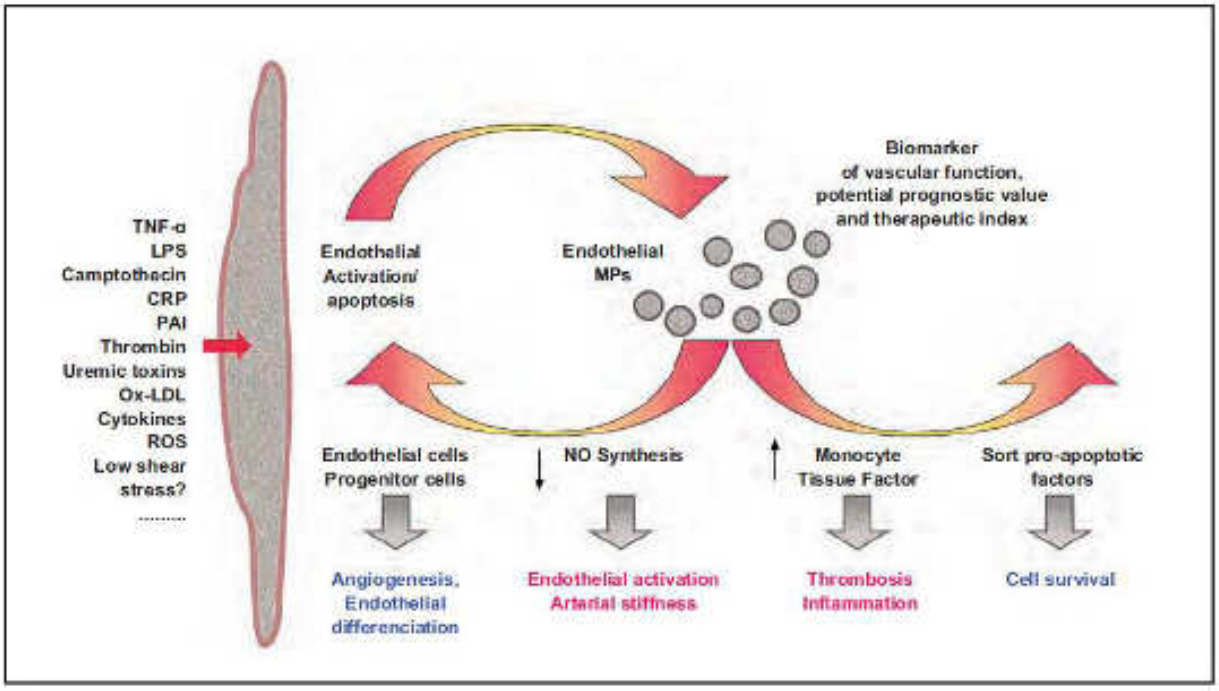

Figure 4. Schematic representation of the different agonists known to augment MP released from cultured endothelial cells and their paradoxical biological functions (Adapted with permission from American Heart Association).

Exosomes (<100 nm in diameter) are produced in multivesicular bodies during endocytosis and they play a role in antigen presentation. Unlike MP, they do not externalize PS and they express specific exosomal markers such as Lamp1, CD63, and TSG101; they also contain RNA and microRNAs (21).

Apoptotic bodies are larger than MP or exosomes and are characterized by externalized PS and, unlike MP, a permeable membrane facilitating propidium iodide staining of the nuclear material they contain (76). Several reports indicate that apoptotic bodies are passive cargos delivering their nuclear content (oncogenes, DNA, microRNA) to phagocytes by horizontal transfer (76,77), and thus they share this specific property with EMP (78).

Besides TNF-α, other inflammatory cytokines and also bacterial lipopolysaccharides, reactive oxygen species (79), plasminogen activator inhibitor (80), thrombin (81), camptothecin (82), C-reactive protein (CRP) (83), and uremic toxins (84) are able to induce *in vitro* EMP generation. Interestingly, endogenous nitric oxide dampens the release of EMP on stimulation with CRP by a mechanism involving tetrahydrobiopterin (83).

Although MP of endothelial origin represent a sparse population of circulating MP, changes in their plasma levels might carry important clinical information in healthy subjects and in patients with cardiovascular disorders (73). In patients presenting a characterized endothelial dysfunction, levels of circulating EMP are inversely correlated with the amplitude of flow-mediated dilatation, independently of age and pressure (85-90). Furthermore, acute endothelial injury such as that induced by secondhand smoke rapidly impairs endothelial function and increases circulating EMP in young healthy subjects (91). Therefore, EMP emerge as a new surrogate marker of endothelial health.

So far, only a few studies have investigated the prognostic potential of the measurement of EMP plasma levels. In patients with acute ischemic stroke, EMP levels are associated with lesion volume and clinical outcome, but there was no report about clinical events during follow-up (92). In patients with pulmonary hypertension, circulating levels of EMP expressing E-selectin predict the 1-year outcome (93). In subjects with high risk of coronary heart disease, baseline levels of EMP expressing vascular endothelial cadherin predicted outcome, independently of Framingham score and of CRP and brain natriuretic peptide (BNP) levels (94). Similar findings were observed in chronic renal failure, where high values of $CD31^{+}CD41^{+}$ EMP were independent predictors of cardiovascular death, whereas other MP plasma subpopulations had no prognostic value (95). These data suggest that EMP levels may be used in the future as a biomarker for stratification of patients and identification of subjects with a high risk of developing cardiovascular complications. Recently, an

interest in multimarker strategies combining EMP with endothelial progenitor cell levels has emerged from the literature as an integrative marker of vascular health. A change in the ratio of EMP to endothelial progenitor cells may reflect an imbalance between endothelial damage and repair that could be useful to identify patients with damaged vasculature (96,97).

The potential contribution of EMP in endothelial cell survival by showing that EMP release could protect endothelial cell apoptosis by diminishing levels of caspase-3 in cultured endothelial cells resulting from trapping caspase-3 in MP (98). Thus, endothelial-derived MP contribute to the sorting of several proapoptotic factors preventing cell detachment and apoptosis

Finally, EMP carrying endothelial protein C receptor and activated protein C (APC) could also promote cell survival by induction of cytoprotective and anti-inflammatory effects (99).

Taken together, the involvement of EMP in vascular homeostasis appears to be more complex than initially thought. EMP can play a major role in inflammation, thrombosis, and angiogenesis. However, depending on the pathological context, the mechanisms and sites of formation, EMP could have favorable effects to maintain vascular homeostasis. These paradoxical functions might result from EMP composition, as proteomic analysis has shown that one third of the proteins found on EMP are specific to the stimulus initiating their release, not only demonstrating the plasticity of these vesicles but also revealing the complexity of the mechanisms governing their formation (100).

MPs in Angiogenesis

Angiogenesis is a tightly regulated process that involves endothelial cell survival, proliferation, migration, differentiation, and morphological changes, such as tube formation. It is a major process in many pathological conditions, such as tumor growth, diabetic retinopathy, and inflammation, as well as in embryonic development and wound healing (99).

Most of the research regarding MP has been focused on MP from blood cell origin and on their angiogenic activity, mainly in the tumor microenvironment. However, MP derived from various types of cells, related to other angiogenesis-associated disorders, were found to have angiogenic properties. Submicron membrane vesicles shed from retinal, vascular, and circulating cells were significantly increased in vitreous fluid of patients with proliferative diabetic retinopathy. These MP, isolated from human vitreous sample of patients, were found to stimulate endothelial cell proliferation and formation of new vessels (100).

MP, derived from human circulating endothelial progenitor cells, was shown to activate an angiogenic program in mature quiescent endothelial cells. Endothelial progenitor cell derived MP expressed several adhesion molecules that were instrumental in MP internalization into endothelial cells and required for their biological activity. The MP-induced antiapoptotic effect organization in capillary-like structures was dependent on mRNA transfer. Also, microarray analysis was pre-formed on MP derived from endothelial progenitor cells, and transcripts associated with the phosphatidylinositol 3-kinase/Akt signaling pathway and with endothelial nitric oxide synthase (known to be involved in the angiogenic and antiapoptotic program) were found (78).

Platelets contain various angiogenesis-related substances that release into the environment upon platelet activation. Moreover, it was recently demonstrated that platelets, as a cellular system, could induce an angiogenic response (101,102). At the same time, platelet activation at sites of blood flow disturbances or endothelium injury results in formation of PMP. Since platelet activation frequently occurs at the sites where angiogenesis takes place (e.g., in the tumor vasculature, or in the proximity of thrombus in an ischemic site), a possible impact of PMP in blood vessel development would be of importance, either as a part of pathogenesis of the malicious processes, or as a counteracting factor.

PMP triggered an angiogenic response, both *in vitro* and *in vivo*. This effect is mediated by intra-particle cytokines, i.e., VEGF, bFGF, and PDGF. Separate inhibition of each cytokine resulted in a significant suppression of the vessel sprouting, which suggests that a mutual action of pro-angiogenic compounds is needed for the development of an angiogenic response (103)

The present study demonstrates for the first time that shed-membrane MPs isolated from human atherosclerotic lesions stimulate endothelial cell proliferation and promote *in vivo* neo-vessel formation after CD40 ligation. The endothelial proliferative effect of plaque MPs was more pronounced when MPs were isolated from symptomatic patients compared with that seen in asymptomatic patients, and this finding was associated with an increased number of $CD40L^+$ MPs in these patients. Therefore, accumulation of MPs in atherosclerotic lesions may represent an endogenous signal for atherosclerotic plaque neovascularization and vulnerability (104).

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MPs in Cardiovascular Disease

There are substantial differences between the fractions of MPs or subpopulations in the blood of healthy subjects and those present in patients suffering from diseases with increased thromboembolic risk or vascular damage, such as atherosclerotic vascular disease, sepsis, diabetes, chronic severe hypertension, and preeclampsia (18,105,106). Accordingly, in patients with acute myocardial infarction, elevated numbers of MPs are present compared with healthy controls (105,106,107). Moreover, subtypes of MPs differ between patients with stable angina and those with acute coronary syndromes or myocardial infarction (108,109).

The clinical relevance of the presence of MPs in the blood of healthy subjects is unclear but can be regarded as a reflection of the dynamics of their production by resting, activated, and apoptotic cells and their clearance. In vascular disease states, it remains to be elucidated whether MPs are a cause or a consequence of the condition because disease-related factors, such as infectious agents, cytokines, and metabolic disturbances, are all known to affect the release of MPs (18,105,106).

In cardiovascular disorders, two distinct pools of MPs appear of interest: (1) circulating MPs released from vascular and peripheral blood cells; and (2) MPs shed by apoptotic cells sequestered within the atherosclerotic plaque and eventually exposed to flowing blood after rupture (110,111).

In acute coronary syndromes, TF triggers the formation of intracoronary thrombi following endothelial injury. The acellular lipid-rich core of an atherosclerotic plaque represents its most thrombogenic part (112), with enhanced TF activity being directly supported by TF^+-MP exposing PhtdSer. Apoptotic macrophages constitute the main source of membrane-bound TF (110,113). Smooth muscle cells (SMCs) may also contribute to TF^+-MPs accumulation in the lipid core. Several mechanisms involving MPs from the plaque could account for instability, as suggested by *in vitro* data. MP would mediate the recruitment of inflammatory cells within the plaque. Endothelial-derived MPs released on VEGF or FGF2 stimulation harbor functional matrix metalloproteinases possibly favoring fibrous cap proteolysis. In the course of plaque remodeling, MPs of various origin could modulate angiogenesis, a key determinant of plaque vulnerability (114).

MPs may not only have deleterious effects by promoting coagulation and inflammation or by modifying endothelial function, which all contribute to the development of Cardiovascular Disease (CVD); but may also have beneficial effects. First, recent studies have shown that MPs are efficient vectors that exchange biological information between cells (intercellular communication) (18,105,106). Second, the release of MPs protects cells against the consequences of external stimuli or stress. Endothelial cells escape from complement-induced lysis by releasing MPs carrying the lytic complement C5b-9 complex (115). Similarly, the release of MPs protects cells against an overshoot in (internal) cellular reactions triggered by external stressors. Regarding the latter, MPs play a role in "cellular waste management" because they contain increased (compared with parent cell) concentrations of chemotherapeutics, oxidized phospholipids, or caspase 3 (18,105,106).

In patients with subclinical or less occlusive atherosclerosis, more endothelial MPs are present when compared with patients with established or symptomatic atherosclerosis (109,116), suggesting that the ability of the endothelium to release MPs depends on its integrity and viability. In other words, if the ability of the endothelium to release MPs becomes impaired or inhibited, the integrity and viability of the cells may deteriorate.

In vitro, MPs from various cellular or disease origins or both induce endothelial dysfunction, especially by altering the balance between NO and reactive oxygen species (ROS) production and release (117-119).

Evidently, MPs are able to restore endothelial injury through their dual ability to increase NO and reduce ROS. In summary, MPs can have both detrimental and beneficial effects on endothelial functions, especially by altering the balance between NO and ROS production and release. It seems that these effects are dependent on the specific stimulus underlying the release of MPs by their parent cells.

In the light of the previously described procoagulant and proinflammatory properties of MPs, together with the association between elevated numbers of MPs and clinical CVD, the prevailing view is that circulating MPs are harmful, contributing to CVD and risk of CVD. However, as previously outlined, in addition to their potentially harmful effects, cell-derived MPs may also be beneficial and protect against cellular and vascular damage. Therefore, it is not surprising that both elevated and lower levels of circulating MPs have been associated with (risk factors of) CVD (120).

Interestingly, elevated platelet MPs were described in patients with both type 1 and 2 diabetes, hyperlipidemia, obesity/metabolic syndrome, and hypertension (18,105).

Thus, in plasma samples from patients with chronic severe hypertension compared with patients with mild hypertension and controls, more MPs exposing platelet endothelial cell adhesion molecule-1 (PECAM-1) (CD31), but not glycoprotein Ib (CD42) (ie, MPs presumably of endothelial or platelet origin or both), were found (121,122). In these patients, elevated numbers of MPs are likely to reflect the cellular stress of endothelial cells and platelets.

Preliminary data indicate that plasma levels of MPs could be of prognostic value for the occurrence of cardiovascular diseases. In a 6-month follow-up study, circulating annexin V+MPs appeared as a robust predictor of the occurrence of secondary myocardial infarction or death in 500 patients with acute coronary syndromes (123). Furthermore, circulating leukocyte-derived MPs, unlike platelet-derived MPs, predict subclinical atherosclerosis burden appreciated by plaque numbers in carotid arteries, abdominal aorta, and femoral arteries in > 200 asymptomatic subjects (124).

Although the prognostic potential of circulating MPs is still in its infancy, the different studies mentioned above clearly demonstrate that their detection and quantification is an interesting and potentially valuable tool to appreciate

MPs in Cancer

MPs have been widely detected in various biological fluids including peripheral blood, urine and ascitic fluids, and their function and composition depend on the cells from which they originate. By facilitating the horizontal transfer of bioactive molecules such as proteins, RNAs and microRNAs, they are now thought to have vital roles in tumor invasion and metastases, inflammation, coagulation, and stem-cell renewal and expansion (125).

MP-mediated cargo transfer to adjacent or remote cells has been shown to affect many stages of tumor progression (126), including angiogenesis, escape from immune surveillance, ECM degradation and metastasis (Fig. 5). MPs shed from tumor cells facilitate transfer of soluble proteins (127), nucleic acids (128), functional trans-membrane proteins (129), chemokine receptors (130), tissue factor (129) and receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) (131,132).

A recent report showed that the oncogenic receptor EGFRvIII, which is found exclusively in a subset of

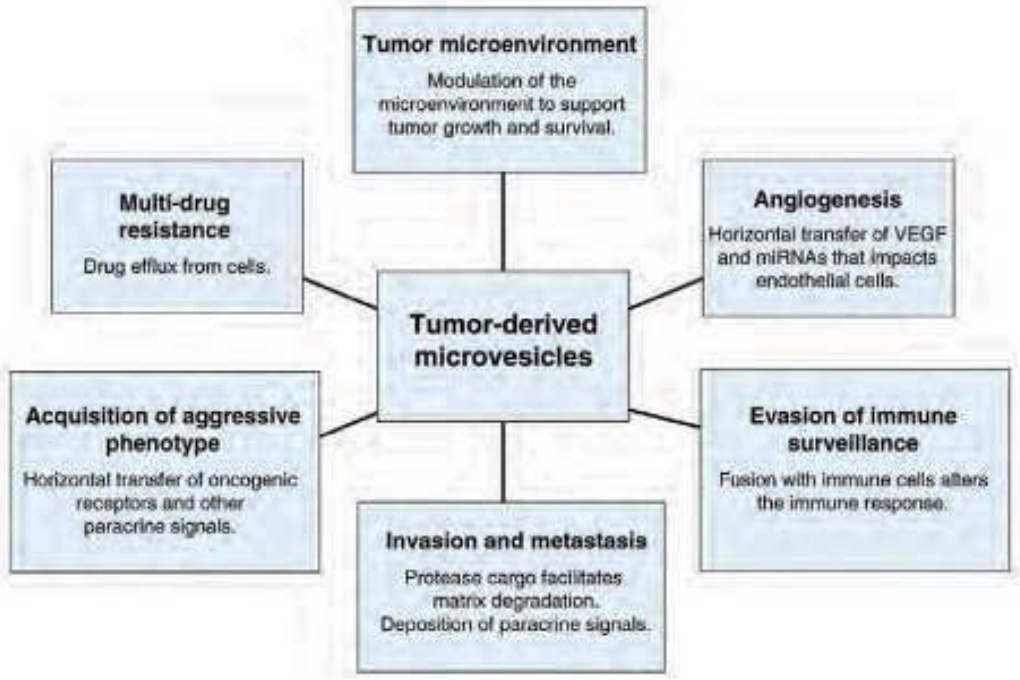

Figure 5. Tumor – derived MPs influence many aspects of cancer progression (Adapted with permission from The Company of Biologists Ltd.).

aggressive glioma tumors, was transferred to a non-aggressive population of tumor cells through MPs (131). As a consequence, the recipient cells exhibited the activation of two signaling pathways [mitogen-activated protein kinase (MAPK) and Akt] and changes in the expression of EGFRvIII-regulated genes [vascular endothelial growth factor (VEGF), Bcl-Xt, p27], leading to morphological transformation and an increase in anchorage-independent growth.

Thus, MPs secreted by tumor cells induce endothelial cells to release MPs that contain VEGF and sphingomyelin in order to promote angiogenesis. It is interesting that in lung cancer models, hypoxia induces an increased release of MPs (133). Thus, the adverse tumor microenvironment somehow triggers tumor cells to release MPs, which in turn facilitates angiogenesis by bringing nutrients and oxygen to the rescue of cancer cells.

A range of hematological complications broadly categorized as 'thromboembolism' is associated with cancer-related mortality (134). A recent study showed that most of the TF-bearing MPs were tumor derived (135). The group further confirmed the association between the presence of TF-bearing MPs and an increased risk of thromboembolic disease in malignancy (135). Additionally, activation of the coagulation system and TF signaling has also been suggested to deliver growth-promoting stimuli to dormant cancer stem cells (136).

Hypothetically, cancer cells can fuse with MPs derived from non-cancer cells to camouflage behind the lipids and membrane-specific proteins of non-transformed cells. A study by Tesselaar and colleagues identified a low number of circulating MPs from cancer patients that stained for both MUC1, a cancer-cell marker, and glycoprotein IIIa, a protein that is exclusively present on platelets (137). It could be argued that such MPs are released by tumor cells after they have fused with MPs released by platelets. All of the above suggest that the horizontal transfer of MP cargo can successfully divert immune cells to altered phenotypes, thereby facilitating cancer-cell evasion of the immune response.

Matrix degradation is essential for promoting tumor growth and metastasis (138). As indicated above, MPs that are shed by tumor cells are loaded with proteases and provide an additional means of matrix degradation, creating a path of least resistance for invading tumor cells. Accordingly, studies report the presence of Matrix Metalloproteinase (MMP)2, MMP9, MT1-MMP and their zymogens urokinase-type plasminogen activator (uPA) and EMMPRIN, within tumor-derived MPs (139-142). Given the importance of matrix degradation in tumor metastases, it is logical to hypothesize that there is a direct correlation between the number of invasive MPs and tumor progression.

An example for the direct involvement of MPs in facilitating tumor-cell survival comes from the demonstrated expulsion of therapeutic drugs from tumor cells through MPs. Tumor cells treated with doxyrubicin accumulated and released the drug in shed MPs, implying MP shedding as a drug-efflux mechanism involved in drug resistance (143). Another study documented that MPs of cisplatin-insensitive cancer cells contained 2.6- fold more cisplatin than cisplatin-sensitive cells that release MPs (44). Therefore, by virtue of their ability to harness select bioactive molecules and propagate the horizontal transfer of these cargoes, shed MPs can have an enormous impact on tumor growth, survival and spread. Molecules that regulate MP shedding and proteins on circulating MPs that are responsible for tumor growth, progression and survival will be effective targets for anti-cancer therapeutics. Tumor-specific markers that are exposed on circulating MPs might be particularly useful as potential biomarkers. The protein composition of MPs might reflect molecular changes in tumor cells from which they are derived and, therefore, can potentially serve as a prognostic indicator of disease stage and efficacy of treatment.

MPs in Hypertension, Diabetes, and Chronic Renal Failure

Severe, uncontrolled hypertension is associated with high rates of target organ complications (145-150), but the molecular mechanisms by which extreme blood pressure elevation leads to vascular injury are not well defined. Increasing evidence suggests that hypertension confers a prothrombic state, characterized by abnormalities of endothelial function and platelet activation (151-168). Consequently, investigative interest has recently focused on endothelial and platelet activation as important mediators of hypertensive vascular injury (121).

Endothelial Cells Microparticles (EMP) release can be caused by a number of cytokines such as interleukin-1 and tumor necrosis factor and by elevated shear pressure (169-174). Assays for circulating EMP have recently been developed (169,170) as potential means of quantifying endothelial cell injury.

Platelet derived Microparticles (PMP) concentration is a marker of platelet activation (14,175-176). PMP are formed by platelet membrane vesicle formation and shedding (14). PMP are known to possess procoagulant

activity and are elevated in severe thrombotic states such as acute myocardial infarction and stroke (14,177-181). EMP and PMP have diverse effects on coagulation, leukocytes, platelets, and endothelium that could ultimately contribute to the pathogenesis of the acute vascular injury observed in patients with uncontrolled severe hypertension. EMP and PMP may therefore be mediators of as well as markers for endothelial and platelet activation and hypertensive target organ injury (121).

Pulmonary arterial hypertension (PAH) is a severe disease of the small pulmonary arteries characterized by vascular narrowing and raised pulmonary artery pressure leading to the development of right-sided heart failure and death. In PAH, vasoconstriction, remodeling of the pulmonary vessel wall, endothelial and vascular smooth muscle cell proliferation and dysfunction, and thrombosis contribute to increased pulmonary vascular resistance (PVR), right ventricle overload, and stretch (182).

Circulating EMPs are increased in chronic renal failure (CRF) and hemodialyzed (HD) patients and represent a new marker of endothelial dysfunction in uremia. In addition, the ability of p-cresol and indoxyl sulfate to increase an EMP release in vitro suggests that the specific-uremic factors could be involved in an EMP elevation in patients (84,86).

Type 2 diabetes is associated with accelerated atherosclerosis (183,184), which is evidenced already early in the course of the disease. Recently, increased numbers of PMP were reported in type 2 diabetic patients with poor metabolic control and microvascular complications (185). TF, possibly of granulocytic origin, is exposed on MP subpopulations in asymptomatic patients with well-regulated type 2 diabetes. TF-positive MPs are associated with components of the metabolic syndrome but not with coagulation. Thus, TF on MPs may be involved in processes other than coagulation, including transcellular signaling or angiogenesis (186).

Compared with age-matched control subjects, type 1 diabetic patients presented significantly higher numbers of platelet and endothelial MPs (PMP and EMP), total annexin V–positive blood cell MPs (TMP), and increased levels of TMP- associated procoagulant activity. In type 2 diabetic patients, only TMP levels were significantly higher without concomitant increase of their procoagulant activity. Interestingly, in type 1 diabetic patients, TMP procoagulant activity was correlated with HbA1c, suggesting that procoagulant activity is associated with glucose imbalance. Thus, diabetic patients differ by the procoagulant activity and the cellular origin of MPs (187).

Endothelial cell dysfunction may contribute to the pathogenesis of multiple sclerosis (MS). Elevations of soluble adhesion molecules intracellular adhesion molecule, vascular cell adhesion molecule, and platelet-endothelial cell adhesion molecule-1 (CD31) have been reported as markers of blood–brain barrier (BBB) damage in MS, but direct assay of endothelium has been difficult. Endothelial dysfunction is evident during exacerbation of MS, evidenced by shedding of EMP expressing PECAM-1 (CD31). The *in vitro* data indicate contribution of one or more plasma factors in endothelial dysfunction of MS (173).

MPs in Stem/Progenitor Cells

Experimental studies have suggested that transplantation of stem and progenitor cells may have a beneficial effect on functional and structural recovery in several organs, including heart, liver, and kidney. The mechanisms underlining stem-cell therapy are still intensely debated. Some studies have suggested an engraftment of stem cells by transdifferentiation or fusion in targeted organs. However, a growing number of evidences indicate that transient cell localization in the injured tissue may be sufficient to favor functional and regenerative events, suggesting the release of paracrine mediators (188-190). Several mechanisms involved in cell-to-cell communication have been identified, including secretion of growth factors, cytokines, surface receptors, and nucleotides (191-194). It has been suggested that MPs actively released from cells may play an important role in cell-to-cell communication (6,59,195,196).

Embryonic stem cells were recently shown to represent an abundant source of MPs, and it was suggested that MPs derived from these cells may represent one of the critical components supporting self-renewal and expansion of stem Cells (32,197). In addition, Ratajczak *et al*. (197) demonstrated that embryonic stem cell–derived MPs are able to reprogram hematopoietic progenitors by a horizontal transfer of mRNA and protein delivery.

It has been suggested that transdifferentiation or plasticity of stem cells may at least in part depend on horizontal transfer of mRNA/proteins from the damaged tissue (197). Conversely, MV-mediated transfer of mRNA/proteins derived from stem cells may induce dedifferentiation of mature cells, triggering a proliferative program that may contribute to the repair of tissue injury (52). MP-mediated transfer of mRNA/proteins derived from stem cells may induce dedifferentiation of mature cells, triggering a proliferative program that may contribute

to the repair of tissue injury (78). The mechanism by which embryonic stem cell MPs (ESMPs) may mediate intercellular signaling could involve the activation of receptors on the recipient cell by ligands in the ESMP. In this manner, ESMPs would be able to carry membrane bound ligands considerable distances from their stem cell origin. Alternatively, ESMPs may be able to mediate signaling by the direct transfer of proteins, RNA, or bioactive lipids to the recipient cell, serving as "physiological liposomes" (193,194). If ESMPs can indeed serve as "physiological liposomes," transferring RNA and proteins to cells, they can perhaps be used to deliver exogenously expressed genes for therapeutic purposes (32,197,198).

ESMPs are capable of transferring a subset of miRNAs to mouse embryonic fibroblasts (MEFs), suggesting a tightly regulated transfer process. Transfer of miRNAs by MPs represents a novel method of paracrine signaling, potentially making MPs important components of stem cell niches. It also opens up the possibility of transferring siRNAs via MPs (198).

Recently, it has been proposed that a dynamic stem cell regulation may occur as result of differentiated cell-stem cell interaction via a MP – based genetic information transfer (25). Progenitor/stem cells may re-direct the behavior of differentiated cells by a horizontal transfer of mRNA shuttled by MPs (28,197) and conversely differentiated cells may influence the stem cell phenotype (25).

Deregibus demonstrated that MPs derived from endothelial progenitor cells may activate an angiogenic program in mature quiescent endothelial cells (78) and that mRNA shuttled by MPs derived from mesenchymal stem cells may induce repair of acute kidney injury (199). Recently, Kostin and Popescu (200) demonstrated that the interstitial cajal-like cells that have been described to be present in the heart (201), communicate with neighbouring cells via shedding of MPs.

Herrera *et al.* found that MPs derived from human liver stem cells (HLSC) induced in vitro proliferation and apoptosis resistance of human and rat hepatocytes. These effects required internalization of MPs in the hepatocytes by an α4-integrin-dependent mechanism, suggest that MPs derived from HLSC may activate a proliferative program in remnant hepatocytes after hepatectomy by a horizontal transfer of specific mRNA subsets (202).

The ability of MPs to transfer RNA and protein, and to act as paracrine factors raises very exciting possibilities for therapeutic uses. Cells engineered to express mRNA, siRNA, or protein may be capable of delivering these macromolecules to local cellular environments via MPs. These engineered cells can be encapsulated to provide sustained local delivery. Since current techniques for gene transfer use viral or synthetic agents as delivery agents, their replacement by MPs released from autologous transplants of engineered cells will offer the advantage of a virus-free approach and make the prospects of gene therapy safer (198).

Conclusion

In conclusion, the scientific community has made considerable progress to date in recognizing MP as important mediators of intercellular communication rather than irrelevant cell debris. We have already learned much about the biological effects of MP. Future steps would be to (i) explore their full potential diagnostic application, (ii) develop efficient strategies that will allow us to modulate their secretion in various clinical situations and finally (iii) employ MP as tools to modify the biological responses of cells. A new era of investigation and opportunity for drug development has begun!

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