This Review is the first of a new thematic series on **Microparticles**, which includes the following articles:

Christian Weber and Sebastian Mause, Guest Editors

Microparticles

Protagonists of a Novel Communication Network for Intercellular Information Exchange

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Abstract: **Microparticles represent a heterogeneous population of vesicles with a diameter of 100 to 1000 nm that are released by budding of the plasma membrane and express antigens specific of their parental cells. Although microparticle formation represents a physiological phenomenon, a multitude of pathologies are associated with a considerable increase in circulating microparticles, including inflammatory and autoimmune diseases, atherosclerosis, and malignancies. Microparticles display an 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 microparticles as veritable vectors for the intercellular exchange of biological signals and information. Indeed, microparticles may transfer part of their components and content to selected target cells, thus mediating cell activation, phenotypic modification, and reprogramming of cell function. Because microparticles 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. At least in same cases, the sequential steps of the transfer process underlie complex regulatory mechanisms, including selective sorting ("packaging") of microparticle components and content, specificity of interactions with target cells determined by surface receptors, and ultimately finely tuned and signal-dependent release and delivery of microparticle content. (*Circ Res*. 2010;107:1047-1057.)**

Key Words: microparticles ■ cardiovascular disease ■ platelet ■ signal transduction ■ vascular biology

It is well established that virtually all eukaryotic cells possess the fundamental capacity to release small vesicles, generally referred to as microparticles (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.[1] Although MPs are present in peripheral blood of healthy individuals, with platelet MPs

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Non-standard Abbreviations and Acronyms	
AA	arachidonic acid
CD	cluster of differentiation
EGFR	epidermal growth factor receptor
EOC	early outgrowth cell
GP	glycoprotein
IL	interleukin
miR	microRNA
MP	microparticle
PPAR	peroxisome proliferator-activated receptor
TF	tissue factor
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor

being the most abundant and representing 70% to 90% of all circulating MPs,[2,3] marked elevations occur in many disease states. These conditions include autoimmune disorders, atherosclerosis, malignancies, and infection among others.[2,4–6] 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 (see Table 1).[7,8] The aim of this review is to highlight the concept of the MPs as potent vectors of biological information and protagonists of an intercellular communication network. Special emphasis is placed on the multifaceted objects of transfer and on the principal consequences of such signal exchange.

Shedding and Composition 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.[2,9,10] The activation-induced formation of MPs is initiated by an agonist-mediated increase of intracellular calcium resulting in an elementary rearrangement of the phospholipid asymmetry with translocation of phosphatidylserine from the inner to the outer surface leaflet of the plasma membrane. This membrane reorganization involves the calcium-dependent inhibition of a specialized flippase named aminophospholipid translocase and the engagement of floppase and scramblase.[5,11,12] A link between the modulated activity of phospholipid transporters and the vesiculation of MPs is highlighted by characteristics of the bleeding disorder in patients with Scott syndrome. Presumably as a consequence of a defect in the scramblase pathway, the outward transmembrane migration of phosphatidylserine but also the potential in shedding procoagulant MPs is dramatically impaired in Scott cells, resulting in an abnormal hemostatic response.[13–15] Concomitant with the collapse of the membrane asymmetry, calcium-sensitive enzymes such as calpain and gelsolin are activated, promoting the detachments of membrane proteins to cytoskeletal structures by proteolytic activity and thus enable subsequent vesiculation.[16] Shedding of MPs might also be a consequence of early apoptosis with remodeling of the submembranous cytoskeleton of apoptotic cells and membrane blebbing. Apoptotic MP formation critically depends on the Rho-associated kinase ROCK I, which is activated during apoptosis via caspase-mediated cleavage.[3,17] This truncated form of ROCK I may promote phosphorylation of myosin light-chains and coupling of actin-myosin filaments to the plasma membrane and may thus trigger cytoskeletal rearrangement and formation of apoptotic MPs as well as redistribution of DNA.[18] Of note, it has been suggested that MPs formed during cellular activation phenotypically and quantitatively differ from MPs released during apoptosis of the same cell type. For instance, a comparison of antigen expression on MPs from microvascular endothelial cells revealed that the endothelial markers CD31 (PECAM-1) and CD62E (E-selectin) are more markedly expressed by MPs released from apoptotic cells, whereas CD51 (integrin α5) and CD54 (ICAM-1) are preferentially expressed by activation-induced MPs.[19] In contrast to MPs, apoptotic bodies are released during the terminal steps of programmed cell death and represent condensed and DNA-rich remnants of the fragmentated apoptotic cells (see Table 1).[17] Such apoptotic bodies, which generally exceed MPs in size (up to 4 μm), may act as passive shuttles delivering DNA and oncogenes to the nuclear compartment of the phagocytosing cell.[20,21]

Table 1. Characteristics of Different Types of Secreted Vesicles

Feature	Exosomes	Microparticles	Apoptotic bodies
Size	50–100 nm	100–1000 nm	Up to 4000 nm
Sedimentation	100 000 *g*	20 000 *g*	16 000 *g*
Origin	Multivesicular, internal compartments	Plasma membrane	Cellular fragments
Release	Constitutive and/or cellular activation	Cellular activation and early apoptosis	Terminal apoptosis
Annexin V binding capacity	No/low Annexin V binding capacity	High Annexin V binding capacity	High Annexin V binding capacity
Marker proteins	Tetraspan protein CD63	Integrins, selectins, other antigens of parental cell	Histones
References	7, 8	2, 3	17, 20

MPs display a variable and abundant spectrum of bioactive substances, membrane-anchored receptors and adhesion molecules on their surface, allowing specific interaction and crosstalk with various target cells. Notably, externalization of phosphatidylserine provides an efficient platform for the assembly of blood coagulation enzymes, culminating in the activation of the coagulation cascade.[22–24] As MP membranes engulf some cytoplasm during membrane blebbing, they also carry a rich set of cytokines, chemokines, enzymes, growth factors, and signaling proteins.[16,25,26] This finding has recently been complemented by the detection of functional mRNA and microRNA (miR) species in certain MPs.[27,28]

Although antigens found on the surface of MPs and the cargo of MPs resemble those of their parental cells (eg, lineage markers), MPs represent more than just a miniature version of the specific cell of origin, as certain MP components are selectively enriched compared to their parental cell and as the composition and the function of MPs not only depends on the cellular origin but also on the agonist responsible for MP formation and the microenvironment of the parental cell.[13,24,29–34] For instance, it has been shown that because of the considerable local concentration of phosphatidylserine and membrane proteins participating in the binding of coagulation factors, the surface of platelet MPs is approximately 50- to 100-fold more procoagulant than the surface of activated platelets.[35] Similarly, the density of other surface molecules originating both from the plasma membrane (eg, β3-integrin) and from translocated intracellular granule membranes, eg, P-selectin, is highly enhanced on platelet MPs, and MPs from activated neutrophils are characterized by a 10- fold enrichment of Mac-1 present in an activated state, compared to MPs from nonactivated neutrophils carrying low levels of Mac-1 in an inactive state.[36] Whereas in some cases the reported enrichment of components in MPs just reflects agonist-induced changes in paternal cells (eg, accumulation of α-granule constituent P-selectin on the surface of activated platelets), it is speculated that under certain conditions indeed inclusive or exclusive sorting mechanism take center stage for the selection of MP components.[11,31,37] Such sorting may inter alia rely on the lateral organization and accumulation of membrane lipids and proteins in cholesterol-rich raft domains.[29,30,38] However, continuative studies are necessary to elucidate the mechanism and the exact determinants of this phenomenon. Demonstrating the relevance of the activating stimulus for the composition of MPs, proteomic analysis demonstrated that different MP populations with overlapping but distinct protein arrangements were generated from endothelial cells by stimulation with plasminogen activator inhibitor type 1 (PAI-1) or tumor necrosis factor (TNF)-α.[32] Likewise, platelet MPs generated in response to dibucaine do not harbor α-granule proteins such as CXCL4, β-thromboglobulin, or fibrinogen as opposed to calcium ionophore A23187–induced platelet MPs.[39] Further accentuating the level of complexity, evidence was provided for a differential function of surface receptors on MPs. For instance, MPs from thrombin- or collagen-activated platelets expose glycoprotein IIb–IIIa complexes that bind fibrinogen, whereas those produced by platelets activated with C5b-9 do not.[13] More generally, it has been shown that circulating MPs isolated from patients with myocardial infarction impaired endothelium-dependent vasorelaxation, whereas MPs from nonischemic patients had no effect on vasomotor function.[40] In summary, the mode of cell activation and the microenvironment where MPs were generated relevantly determines the nature of MP-mediated intercellular communication by controlling the composition of MPs.

Table 2. Microparticles As Transcellular Delivery Systems for Biological Signals and Information

Object of Transfer	MP Donor Cell → Target Cell	Reference(s)
Receptors/surface molecules		
CCR5	PBMCs → various cells	50
CXCR4	Platelets → various cells	51
	Platelets → EOCs	54
GPIIb/IIIa	Platelets → neutrophils	58
	Platelets → EOCs	59
EGFR	Tumor cells → various cells	38
MHC molecules	Immune cells → immune cells	43, 44
TF	Monocytes → platelets	30
Intraparticular proteins		
Cytokines/ chemokines		
IL-1β	Various cells → various cells	62, 63, 64, 65
RANTES/CCL5	Platelets → endothelial cells	42
Growth factors		
VEGF	Platelets → endothelial cells	67, 68
	Tumor cells → endothelial cells	70
bFGF	Platelets/tumor cells → endothelial cells	67, 68
PDGF	Platelets → endothelial cells	68
Proteases		
MMPs	Tumor cells → extracellular matrix	70
EMMPRIN	Tumor cells → extracellular matrix	72
caspase 1	Monocyte → SMCs	71
Others		
PPARγ	Platelets → monocytes	66
RNA		
mRNA	Stem cells and others → various cells	27, 75
miR	Stem cells and others → various cells	28, 37, 74, 55
Lipids		
AA	Platelets → various cells	85, 86, 87
PAF	Various cells → platelets and others	88, 89

MP-Based Transfer of Biological Information

Whereas MPs were originally considered as inert cellular debris, numerous studies provided now a rationale for the concept of MPs as veritable vehicles for the intercellular exchange of biological signals and information (see Table 2).[8,41] Principally, 2 main mechanisms by which MPs mediate

intercellular signaling may be discerned. First, MPs may act as circulating signaling modules affecting cellular properties and responses by activation of receptors on the target cell via presentation of membrane-associated, bioactive molecules. Second, MPs may mediate signaling by directly transferring part of their content or components including proteins, bioactive lipids or RNA to the recipient cell, potentially resulting in cell activation, phenotypic modification and reprogramming of cell function. This transfer may be sufficiently facilitated by transient interactions, or may require firm association, membrane assimilation or definitive incorporation of MPs into the target cell.[30,42–44] Because MPs not only are found in the vicinity of the activated parental cell but also readily circulate in the vasculature, both mechanisms allow signaling not only to neighboring cells but also to cells at remarkable distance from their donor cell origin. Recent studies even raised the possibility that CNS-derived vesicles may enter the bloodstream and interact with endothelial cells in the peripheral circulation, representing a novel communication channel between the nervous system and the cardiovascular system.[45] Based on their ability to transfer part of their components and content to target cells, MPs quantitatively and qualitatively complement traditional methods of intercellular communication such as direct secretion of signaling molecules, physical interaction of membrane proteins and involvement of gap junctions.[46] This MP-based transcellular delivery system establishes an integrated communication network in which specific properties and information among cells can be rapidly shared and complex processes such as immunoregulation and maintenance of vascular hemostatic balance can be more efficiently coordinated. Notably, under certain pathophysiological conditions, MPs may thus also perpetuate and aggravate deleterious local and general processes such as inflammation, thrombosis, and metastatic spread of cancer.[47,48]

Two key factors contribute to the remarkable functional activity and relevance of the MP-based communication system: the opulence of the MP content with the concentration of bioactive molecules in a small single packet and the compact armament with surface molecules. These surface molecules may ensure in particular a higher specificity and efficiency of the transfer system, as they serve not only as adhesion molecules facilitating specific interactions with selected target cells but may also act as signaling molecules regulating the release of the MP content. For instance, in the context of delivery of the chemokine RANTES/CCL5 to inflamed endothelium, P-selectin and GPIb on platelet MPs mediate transient interactions with endothelial cells, thus arranging a higher frequency of interactions with the endothelial surface which culminates in considerable CCL5 deposition.[42] Furthermore, outside-in signaling mechanisms involving GPIIb/IIIa on MPs are additionally operative in CCL5 release and transfer. Similarly, incorporation of MPs is dependent on their expression profile of surface receptors and on the type and activation status of the target cells.[49] Of note, little is known about the determinants and conditions of MP clearance, but it is suggested that many interactions of MPs with other cells also represent a mechanism of their elimination from the circulation.[5]

The synopsis in the Figure highlights and exemplifies the different modalities and molecular entities that are part of the repertoire that MPs can transfer to elicit their profound effects on selectively responsive targets cells throughout the circulatory system, such as vascular endothelial cells, leukocytes, tumor or progenitor cells.

Transfer of Membrane-Associated Receptors

The transfer of membrane-anchored receptors by MPs results in phenotypic alteration of the recipient cell, thus rendering these cells susceptible to novel interactions and enriching the responses that can be elicited by a given cell type. Mack et al demonstrated that the chemokine receptor CCR5 can be released through MPs from the surface of CCR5-expressing Chinese hamster ovarian cells and peripheral blood mononuclear cells (PBMCs).[50] After coincubation of CCR5-bearing MPs with CCR5-negative PBMCs, monocytes or T cells, CCR5 became detectable on the surface of these cells as determined by flow cytometry analysis. Unveiling a relevance beyond simple physical association, it was shown that the transfer of CCR5 enables binding of the macrophage-tropic HIV-1 to the surface of primarily nonsusceptible cells and to subsequently facilitate internalization of viral particles. Similarly, functional transfer of CXCR4 by MPs of megakaryocytic lineage was shown to contribute to the spreading of HIV, because shuttled CXCR4 serves as a coreceptor for lymphotropic HIV strains.[51] By acquisition of virus coreceptors, cell types such as endothelial cells and cardiomyocytes, which are not primary targets of HIV-1 infection, may become receptive to HIV-1 infection and may play a relevant role in the chronic persistence of the virus infection. Importantly, physical integration of MP-derived receptors into the plasma membrane of the recipient cells may not necessarily confer novel functions. For instance, stimulation with the CCR5 ligand CCL5 induced downregulation of transferred CCR5 in macrophages but not in T cells, suggesting that functional integration of chemokine receptors may be restricted to certain cell types. This clearly warrants further studies to evaluate the determinants and mechanisms by which acquired receptors link to the respective signaling machinery (as a requirement of a functional transfer).

Because a striking overlap between the functional involvement of CCR5 and CXCR4 in cell entry of HIV and the immunopathogenesis of atherosclerosis and vascular repair could be identified,[52] MP-based transfer of these chemokine receptors may be of relevance for the involvement of inflammatory cells and circulating progenitor cells in vascular lesion formation and regeneration after vascular injury. Indeed, platelet MPs were found to amplify the vasoregenerative potential of angiogenic early outgrowth cells (EOCs) (also known as endothelial progenitor cells)[53] after arterial injury and influence the surface expression of CXCR4 on EOCs through different sequential mechanisms.[54] These include receptor transfer, alteration of receptor in- and externalization to and from internal stores, and modified gene regulation. Applying scanning electron microscopy to detect exclusively MP-derived CXCR4 via immunogold labeling, direct membrane assimilation as one mechanism of CXCR4 transfer could be clearly identified. However, confocal mi-

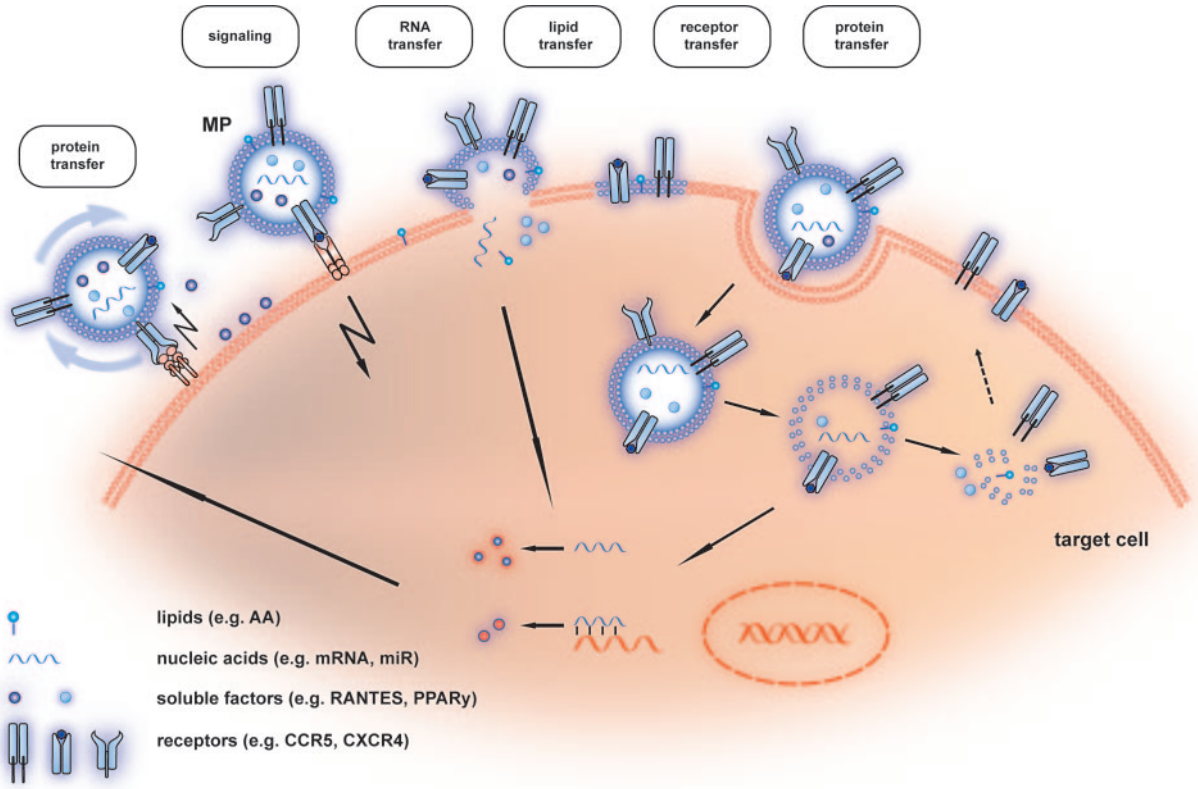

Figure. Repertoire of different molecular constituents and pathways used by microparticles for intercellular transfer of information. Soluble mediators released by MPs (eg, RANTES and IL-1β) can be delivered to target cells during transient or firm interactions and membrane-associated molecules may induce specific responses in target cells. MPs may further transfer membrane components (eg, AA), receptors (eg, CXCR4 and CCR5), and cytosolic and nucleic acids (eg, mRNA and miR) to the target cell by membrane fusion or following internalization. Some content of engulfed MPs (eg, PPARγ or miR-126) induces reprogramming of target cells, whereas membrane-associated MP constituents may be partly recycled and presented on the surface of the target cell.

croscopy analysis also revealed progressive incorporation of MPs into various target cells.[38,54,55] Therefore, a complimentary mechanism of receptor transfer may include fusion of membrane fragments from engulfed MPs with the endosome membrane followed by rapid recycling of CXCR4 to the surface of EOCs. Interestingly, platelet MPs also appear to sensitize CXCR4 on EOCs, resulting in enhanced CXCL12-induced activation of the AKT signaling pathway as well as increased migration and adhesion of EOCs toward CXCL12.[54] By boosting CXCR4-dependent signaling, platelet MPs may stimulate EOC functions in a CXCL12-rich environment, eg, at sites of vascular injury with abundant presence of CXCL12-bearing platelets and smooth muscle cells. Receptor sensitization may be triggered by involvement of sphingosine-1-phosphate, which is substantially harbored by platelet MPs and known to augment CXCR4-mediated signal transduction.[56] Furthermore, platelet MPs may transfer CCL5, which, like other CCR5 ligands, was shown to amplify CXCL12-mediated and CXCR4-dependent signaling processes (eg, Akt phosphorylation) in $CD34^+$ cells.[57]

Of note, receptor transfer as a novel regulatory mechanism is not limited to members of the chemokine network, as it has been shown that platelet MPs may transfer GP IIb/IIIa to neutrophils. These acquired receptors cluster with CD18 (β2-) integrins on the neutrophil surface and are functional, allowing for NF-κB activation in GM-CSF stimulated neutrophils that interact with fibronectin.[58] Recently, it has been proposed that some characteristics used to monitor the endothelial properties of "endothelial progenitor cells" (eg, CD31, von Willebrand factor, lectin staining) may partly rely on an uptake of platelet MPs with concomitant receptor transfer to mononuclear cells during initial cell culture with residual platelet contamination.[59] By using liquid chromatography–tandem mass spectrometry, such cultured "endothelial progenitor cells," referring to angiogenic early outgrowth cells, have been shown to subsequently release MPs containing platelet-derived receptors and integrins (eg, GP IIb/IIIa), although mRNA for these platelet proteins was not present in these cells. Moreover, MPs derived from aggressive glioma cells have been shown to transfer a truncated and oncogenic form of the epidermal growth factor receptor, known as epidermal growth factor receptor (EGFR)vIII, to tumor cells expressing a wild-type receptor. This results in the propagation of oncogenic activity, such as activation of transforming signaling pathways, changes in expression of EGFRvIII-regulated genes (eg, vascular endothelial growth factor [VEGF]) and modulation of proliferative properties of target cells.[38] Thus, MPs from tumor cells may contribute to the dissemination of oncogenes and their associated transforming phenotype among subsets of malignant cells. Notably, application of annexin V derivatives blocking phosphatidylserine mediated interactions have been shown to exert antiangio-

genic effects and inhibit tumor growth in vivo, an effect which might at least in part be attributable to the inhibition of MP-mediated transfer of EGFR to endothelial cells, which was shown to trigger expression of VEGF and autocrine stimulation of VEGFR-2.[60] Similarly to exosomes,[8,61] MPs released from the plasma membrane of mature dendritic cells may modulate local or distant adaptive immunologic responses by transferring major histocompatibility complex (MHC) molecules to resting dendritic cells (via membrane fusion), thereby allowing them to present alloantigens to T cells.[43] Likewise, MPs from ovine B cells were shown to confer antigen-presentation capabilities to bovine polymorphonuclear leukocytes by shuttling MHC class II molecules, thus propagating the capacity to induce a proliferative response and increased cytokine gene expression in alloreactive bovine T cell lines.[44] Beyond the well known procoagulatory function of platelet MPs, monocyte-derived MPs might contribute to the initiation of coagulation by transferring tissue factor (TF) to activated platelets and thereby increase the proteolytic activity of the TF-VIIa complex. Indeed, MPs from blood monocytes or the monocytic cell line THP-1 can arise from lipid rafts selectively enriched with both TF and PSGL-1 (P-selectin glycoprotein ligand-1) but not CD45, and can fuse with activated platelets by an annexin V and PSGL-1 dependent mechanism.[30]

MP Shedding As a Secretory Pathway for the Release of Proteins

Beside membrane-anchored receptors, MPs may transfer proteins such as cytokines, chemokines and growth factors to target cells, resulting in modulation of the constitutive properties of the respective cells. At least in some cases, evidence has been provided for complex regulatory mechanisms during the sequential steps of the transfer process, beginning with the selective (inclusive and exclusive) protein sorting and "packaging" during MP formation, followed by specific interactions with target cells determined by the equipment of MPs with surface receptors and ending with the finely tuned release and delivery of their cargo.

Several studies revealed the presence of the proinflammatory cytokine interleukin (IL)-1β in MPs and supported the concept of MPs as a highly efficient and possibly preferred delivery system for this cytokine. For instance, activation of P2X7 purinergic receptors by extracellular ATP has been shown to induce shedding of MPs from various cells, including monocytic cells, microglia and mature dendritic cells and IL-1β released under these conditions preferentially appeared within the MP fraction rather than in the MP-free supernatant.[62–64] At least in microglia-derived MPs, IL-1β is "packed" into MPs as pro-IL-1β which is subsequently cleaved in the active form by proteases (eg, caspase-1) concomitantly present in MPs. In addition to induce MP formation, ATP secreted by neighboring cells (eg, astrocytes) may also mediate liberation of IL-1β from these MPs via stimulation of P2X7 receptors on MPs. During fibrin clot formation or stimulation with strong agonists, platelets have been shown to translate mRNA for IL-1β into protein and release IL-1β enriched MPs, which may induce upregulation of adhesion molecules and chemokines and trigger endothelial adhesiveness for neutrophils.[65] Similar to IL-1β, the α-granule constituent CCL5 has been shown to be redistributed to platelet MPs on platelet activation and may be transferred to the surface of inflamed and atherosclerotic endothelium to promote subsequent monocyte recruitment.[42] Blockade or deficiency of PMP-expressed adhesion receptors demonstrated that this transfer is a highly regulated process requiring not only transient interactions of MPs with the endothelium with involvement of P-selectin and GPIb, but also outside-in signaling facilitated by GPIIb/IIIa and junctional adhesion molecule-A. These processes may ensure that CCL5 is only released by MPs when in close temporospatial relationship with their specific target cells, thereby allowing rapid capture and effective presentation of CCL5 on its endothelial binding sites and avoiding dilution of the chemokine in the bloodstream. As a result, CCL5 originating from activated platelets is more efficiently transferred to the endothelium when secreted via MPs rather than directly released in a soluble form. Furthermore, it has been shown that peroxisome proliferator-activated receptor (PPAR)γ derived from activated platelets and complexed with the retinoid X receptor is substantially associated with platelet MPs.[66] Interestingly, internalization of PPARγ-containing MPs by monocytic cells elicits a transcellular attenuation of cell activation in the presence of the PPARγ agonist rosiglitazone, strongly suggesting a functional transfer of the transcription factor PPARγ by these MPs. Recent studies supported the concept that MPs participate in angiogenesis by carrying relevant amounts of proangiogenic factors. Indeed, platelet MPs have been shown to induce survival, proliferation and migration of endothelial cells in vitro and injection of platelet MPs into the myocardium may foster postischemic neovascularization after chronic ischemia in vivo.[67,68] Both lipid components and growth factors present in platelet MPs, such as VEGF, basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) have been implicated in these effects, as they may be transferred to target cells.[67,68] In addition, tumor-derived MPs shuttle bFGF and promote invasiveness of endothelial cells by carrying and exposing matrix metalloproteinases (MMPs), such as MMP-2 or MMP-9, which regulate focalized proteolytic activity.[69,70] A recent in vitro study demonstrated that under certain conditions, MPs derived from LPS-stimulated but not from quiescent monocytes may induce apoptosis of target cells by delivering caspase-1 as an effective "cell death message."[71] Of note, in some cases the interaction of MPs with target cells appears to be less controlled and MP-based transfer of biological active molecules is rather a passive than an active and highly regulated process. For instance, it has been shown that the extracellular matrix metalloproteinase inducer EMMPRIN (CD147) may be released from the surface of certain tumor cells via MP shedding.[72] However, the structure of these MPs appears fragile in this specific microenvironment and MPs are rapidly degraded to release bioactive EMMPRIN. In such a scenario, extracellular matrix degradation and hence possibly tumor invasion and metastasis is supported through the microvesicular release of EMMPRIN from tumor cells. A possible trigger for the rather uncontrolled release of content from tumor-derived MPs may be an

acidic pH, which constitutes a common characteristic of the tumor microenvironment. Indeed it was shown in vitro that an acidic pH induced disruption of tumor-derived MPs, resulting in the local liberation of carried VEGF.[70]

Altogether, these findings reveal that MP shedding is a relevant pathway for the rapid release of bioactive proteins, representing a complementary and effective mode of cell communication.

Exchange of Genetic Information by Transfer of RNA

By unveiling that MPs carry substantial amounts of intact mRNA and miR, recent studies identified the intriguing possibility that cells can alter the expression of genes in neighboring and distant cells by transferring genetic information. Following receptor-mediated interactions with target cells and subsequent internalization, MPs may thus extend the confines of the transcriptome, reprogram the phenotype of target cells and confer acquisition of specific features of the donor cell in a variety of conditions. MP-induced modulation of the developmental program of recipient cells may be in particular relevant in case of tissue or cell injury with subsequent initiation of repair mechanisms and involvement of stem and progenitor cells.[73] Indeed, it has been suggested that MPs derived from injured or apoptotic tissue or from cells stimulated during injury (ie, platelets) might mediate increased recruitment and induce phenotypic and functional changes of stem/progenitor cells and thus improve their vasoregenerative potential.[74] Conversely, MPs released from stem/progenitor cells attracted to sites of tissue injury may induce a survival and alarm program of vital residential cells to propagate tissue regeneration.

Importantly, microarray analysis and quantitative RT-PCR indicated that in analogy to proteins and surface receptors, MPs carry a specific subset and not a random sample of cellular mRNA or miR and that only a selected part of the harbored RNA is effectively transferred to recipient cells.[27,37,75] Thus, the message transferred to target cells does not simply reflect the transcriptional status of the donor cell.[76] The mechanisms for this notable selectivity remain to be elucidated, but it is speculated that the "packaging" of MPs with RNA also depends on the activating stimulus and that nucleases present in the recipient cell regulate levels of transferred RNA.[55]

MPs derived from endothelial progenitor cells have been shown to carry a broad range of transcripts and to exert angiogenic activity in primarily quiescent endothelial cells with promotion of endothelial cell proliferation, organization in capillary-like structures and inhibition of apoptosis.[75] Transfer of mRNA with subsequent functional translation could be indeed revealed by transduction of GFP protein in endothelial cells treated with MPs carrying GFP-mRNA, and application of RNase was shown to inhibit MP-mediated activation of the PI3K/Akt signaling pathway and subsequent mediation of proangiogenic effects. Similarly, it has been revealed that MPs derived from embryonic stem (ES) cells harbor a highly enriched and selective cocktail of mRNA as compared to parental ES cells.[27] Together with transfer of various proteins, horizontal delivery of this mRNA cargo to hematopoietic progenitor cells supports their survival as well as expansion and increases their pluripotency.[27] Moreover, MPs derived from liver stem cells may shuttle specific mRNA subsets to hepatocytes and induce proliferation and resistance to apoptosis. These effects require engagement of α4-integrins and subsequent incorporation of MPs in targeted cells. Proving the principle and demonstrating the relevance of MP-exported mRNA, pretreatment of MPs with RNase abrogated MP-induced acceleration of morphological and functional reconstitution of the liver in an in vivo model of partial hepatectomy.[77] Likewise, injured lung cells may use MPs as a vehicle for mRNA and transcriptional regulators to induce epigenetic modifications of cocultured bone marrow cells adopting phenotypic features of lung cells.[78]

In addition to mRNA, it has been recently reported that MPs carry and shuttle a broad range of premature and mature miRs.[28] MiRs constitute a class of highly conserved noncoding RNAs (21 to 24nt) which are partially complementary to multiple target RNAs and control gene expression by either silencing the translation of mRNAs or by inducing faster mRNA-degradation, and thus participate in the regulation of diverse cellular processes, including hematopoietic differentiation, proliferation, apoptosis, hematopoiesis, carcinogenesis and angiogenesis.[79,80] Altogether, it is estimated that more than 30% of human genes are targeted by miRs.[81] The most abundantly expressed miR in plasma MPs was shown to be miR-223,[28] which is known to regulate the maturation, proliferation and differentiation of myeloid and lymphoid cells, may fine-tune the inflammatory response of granulocytes and affects cardiomyocyte glucose metabolism.[82–84] Interestingly, stem cell–derived MPs appear to carry not only various miR, but also a set of ribonucleoproteins, which are known to regulate the intracellular traffic and compartmentalization of RNAs.[37] It remains to be elucidated whether these ribonucleoproteins critically facilitate the selective accumulation of certain mRNAs and miRs in MPs. Recently, it could be shown that apoptotic bodies and MPs from apoptotic endothelial cells are generated and released into the circulation during atherosclerosis and communicate protective activation signals to vascular cells to elicit expression and secretion of the chemokine CXCL12.[74] MiR-126, which is selectively enriched in these vesicles and can be transferred to target cells, could be identified as the main protagonist, as it suppresses the inhibitory function of RGS16 (regulator of G-protein signaling 16) and thereby unleashes CXCR4 to trigger an autoregulatory self-amplifying feedback loop, resulting in increased production and release of atheroprotective CXCL12. Highlighting the function of MPs as regulators of tissue repair and blood vessel homeostasis, administration of these miR-126 enriched MPs conferred beneficial effects on diet-induced atherosclerosis with limited plaque size, increased atherosclerotic plaque stability and enhanced luminal recruitment of Sca-1$^+$ progenitor cells. Furthermore, it has been demonstrated that MPs released by embryonic stem cells can shuttle a subset of miRs to embryonic fibroblasts and thus alter their gene expression.[55] By transferring mRNA and miR, embryonic stem cell MPs may constitute important mediators of signaling within stem cell niches.

Transfer of Bioactive Lipids

In pioneering studies, Barry et al demonstrated that MPs can alter cellular functions through transcellular lipid metabolism.[85–87] Concretely, it was shown that platelet MPs induce platelet activation by the concentrated transcellular delivery of arachidonic acid (AA) and its subsequent metabolism to thromboxane A2, a strong platelet agonist and vasoconstrictor.[85] In addition, the AA fraction of platelet MPs may modulate endothelial cell function by activating a membrane-linked signaling cascade that culminates in expression of cyclooxygenase (COX)-2, which, in turn, may process the transferred arachidonate to bioactive prostanoids such as prostacyclin (PGI 2).[87] Furthermore, it was shown that platelet MPs increased chemotaxis and adhesion of monocytes to endothelial cells, an effect which could be mimicked by AA isolated from platelet MPs. Augmented cellular adhesiveness correlated with AA-induced upregulation of adhesion molecules, such as ICAM-1 (intracellular cell adhesion molecule-1 on endothelial cells and LFA-1 (lymphocyte function–associated antigen-1) and Mac-1 (macrophage antigen-1) on monocytes.[86] Besides AA, platelet-activating factor (PAF) and PAF-like lipids are associated with endothelial and neutrophil MPs and may be subject to transcellular transfer.[88,89] PAF in MPs from endotoxin-challenged PMNs may activate surrounding platelets and thus provide another molecular link between acute inflammation and thrombosis. As mentioned, MPs may propagate angiogenesis via diverse mechanism including transfer of proangiogenic proteins and mRNA, exposure of metalloproteinase activity, and interaction with proangiogenic EOCs. Interestingly, treatment of platelet MPs with activated charcoal, a procedure known to remove lipid growth factors, significantly reduced the angiogenic activity in vitro.[67] This suggests a relevant contribution of MP-derived lipid components, eg, sphingomyelin and sphingosine 1-phosphate which are harbored by MPs and have emerged as players not only in angiogenesis but also in cardiovascular function and atherosclerosis.[90–92]

MPs As Circulating Signaling Modules

Beside their function as transfer vehicles, MPs may act as circulating signaling modules by exposing bioactive, membrane-bound molecules to induce specific responses in target cells.

Of note, in such scenario the facilitated signaling does not require physical transfer of MP components to the target cell. Exemplarily, circulating platelet MPs may exert a regulatory role in the initiation and propagation of adaptive immune responses by serving as concentrated carriers of CD40L (CD154).[93] Remote from the site of platelet activation, these MPs may convey activation signals to the B-cell compartment to augment antigen-specific IgG production and germinal center formation through cooperation with $CD4^+$ T cells. In atherosclerotic lesions, CD40L exposing plaque MPs may stimulate endothelial cell proliferation and thereby promote intraplaque neovascularization and plaque vulnerability.[94] Similarly, tumor-derived MPs may serve as a circulating carrier for Fas ligand (FasL, CD95 ligand), which is known to induce apoptosis of sensitive human lymphoid target cells via interaction with the Fas receptor.[95,96] Shedding of FasL-carrying MPs may thus provide a effective mechanism for long-range signal-directed apoptosis, given the higher efficiency in triggering Fas-mediated apoptosis of membrane associated FasL as compared with its soluble form. These studies substantiate the concept that the formation of MPs allows cells to carry the concentrated activity of membrane-associated molecules away form the site their activation and to transduce signals to distant and selectively responsive targets cells throughout the circulatory system. MPs may also participate in the dissemination of biological signals by exposing enzymes on their surface and therefore induce the activation or shedding of bioactive mediators. For instance, the membrane of endothelial MPs may serve as a surface for the generation of plasmin by expressing urokinase-type plasminogen activator and its receptor. By propagating proteolytic activity within the circulation, these endothelial MPs may gain relevance in linking and regulating fibrinolytic, angiogenic and inflammatory processes.[97] Similarly, platelet MPs present the catalytically active protein disulfide isomerase (PDI) on their surface,[98] which may contribute to the activation of cryptic TF and act as an injury response signal triggering activation of fibrin and thrombus formation following vascular injury.[99] Furthermore, circulating plasma MPs were found to express CD39 and exhibited classic E-NTPDase ectoenzymatic activity to possibly confer antiinflammatory and immunomodulatory properties,[100] and atherosclerotic plaque MPs carry the mature form of TNF-α–converting enzyme (TACE) (also known as ADAM-17) on their surface and may therefore contribute to the alteration and expansion of inflammatory signals in human atherosclerotic by facilitating the shedding of TNF and its receptor TNFR-1.[101]

Conclusion

In the last 2 decades, evidence has been provided that MPs constitute protagonists of a communication network for the local and systemic intercellular exchange of biological information. However, many questions are still unresolved and need to be addressed in the future, including the process of "MP packaging," the underlying mechanism for the observed promiscuity of action of MPs, and the determinants of MP clearance. Further unraveling of the complexity of this network, with its multifaceted intrinsic regulatory mechanism, may open unexpected avenues for the development of novel therapeutic and diagnostic strategies. In this scenario, engineering of genetically modified MPs from selected donor cells with a selective receptor arsenal and desired protein or RNA cargo may be a promising approach. Such an artificially designed carrier system could meet the challenge for an efficient and selective drug transfer because they may allow delivery to a specific location and/or specific cell type (target destination), as well as protect fragile drug molecules from early degradation and denaturation. MPs as therapeutic tools could be particularly useful and relevant for the prevention of accelerated atherosclerosis, as seen after vascular injury with subsequent neointima formation, for the regulation of neovascularization in ischemic tissue (eg, after mycoardial or peripheral ischemia), and for the treatment of cancer. 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.

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None.

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