Microparticles in Cancer

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ABSTRACT

Microparticles (MP) are vesicular structures released from cells upon activation, malignant transformation, stress, or death. MP may be derived from the plasma membrane (shed microvesicles), produced by endosomal pathway (exosomes), or arise from membrane blebs of apoptotic cells. The terms *microparticles* or *microvesicles* (MV) are often used as general and interchangeable descriptors of all cellular vesicles, but a more rigorous terminology is still to be established. The cargo of MP/MV consists of proteins, lipids, and nucleic acids (DNA, mRNA, microRNA), all of which may be transferred horizontally between cells. In cancer, oncogenic pathways drive production of MP/MV, and oncoproteins may be incorporated into the cargo of MV (oncosomes). Oncogenic pathways may also stimulate production of MP/MV harboring tissue factor and involved in cancer coagulopathy. In addition, the cargo of MV may include several receptors, antigens, bioactive molecules, and other species capable of stimulating tumor progression, immunotolerance, invasion, angiogenesis, and metastasis. MP emanate not only from tumor cells but also from platelets, endothelium, and inflammatory cells. Indeed, circulating MP/MV harbor molecular information related to cancer-related processes and may serve as a reservoir of prognostic and predictive biomarkers to monitor genetic tumor progression, angiogenesis, thrombosis, and responses to targeted therapeutics.

KEYWORDS: Microparticles, cancer, oncogenes, tissue factor, microvesicles, exosomes, coagulation, angiogenesis

CELLULAR MICROPARTICLES AS UNITS OF BIOLOGICAL INFORMATION

In a multicellular organism, biological functions are executed by assemblies of cells whose actions must be coordinated by intercellular communication. In this regard, the exchange of signals is usually ascribed to specific molecules (soluble or immobilized) and their corresponding cognate receptors. Such exchange may entail a direct cell-to-cell contact (adhesion, juxtacrine interactions) or release and gradient forming soluble (paracrine) mediators, which may also circulate in blood and body fluids and act in a regional or systemic (endocrine) manner. Such information translates into activation of intracellular signaling networks,[1,2] in either one or two directions,[3] thereby changing the cellular behavior. In addition to these canonical processes, a seemingly direct uptake of factors, enzymes, and particles has also been described in several instances and found to trigger rearrangements of the intracellular machinery.[4–7] Numerous pathways of (uni)molecular intercellular communication have been characterized to date[8] and documented to act as important players in health and disease, including cancer.[9–11]

In addition to their participation in the intercellular networks of the (uni)molecular messages just mentioned, cells may also compose and receive more complex

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(multi)molecular signals, packaged in fragments of plasma membrane, or specialized extracellular organelles of a vesicular (microvesicles),[12,13] tubular (nanotubes),[14] or filopodial (cytoneme)[15] nature. These structures serve to transmit their protein, lipid, or nucleic acid cargo through a process often referred to as *trogocytosis,* or cellular synapse.[16,17] This mode of intercellular contact allows the transmission of both soluble and insoluble (e.g., membrane bound) entities, in a preprogrammed, combinatorial and efficient manner, and while protecting the signaling molecules from extracellular degradation.[18] As in the case with soluble mediators, the vesicular material emitted by various cells may participate in short-, medium-, and long-range communication or in cellular defense and attack processes, whose nature and scope still remains to be understood in a far greater depth.

There is a marked biological diversity among cellular vesicles in terms of their origin, structure, function, and cargo. Therefore, several terms are used to describe them, including microparticles (MP), secretory vesicles (SV), microvesicles (MV), ectosomes (ECSM) or exosomes (EXSM), and many more, depending on the context in which they are studied and their particular properties.[13,18–26] In this article we collectively refer to vesicles released from various cellular sources, including cancer cells, as microparticles (MP) or microvesicles (MV), which are perhaps the most commonly used terms at the moment but not necessarily the most precise. Indeed, in this area a wider consensus as to the nomenclature is still to be reached.[13] Examples of confusing differences in the usage of various terms include description as microvesicles (MV), as either a more general and all-encompassing descriptor of all vesicles, microparticles, and exosomes[23,26] or in a more circumscribed manner. In the latter case, MV are described as plasma membrane–derived structures produced by viable cells, and often referred to as ectosomes.[19] Still, in other publications, microparticles (MP) are viewed mainly as products of cellular apoptosis,[27] and there are sources in which this term is used interchangeably with MV and ECSM.[26] Because different studies use different criteria to describe various microvesicles, their comparisons may be difficult and the nomenclature often inconsistent.

Differences among various MV/MP in terms of their nature and nomenclature may, to some extent, be derived from their cellular sources. In this regard some studies examined circulating MP for lineage/tissue-specific markers to pinpoint their cells of origin. This resulted in the description of distinct MP emanating from platelets (PMP), monocytes (MMP), endothelial cells (EMP), or tumor cells (TMP), which exhibit both similarities and differences.[20,26,28–31] The differential nomenclature of MP may also result from traditions adopted by different research fields, in which processes of cellular vesiculation have been studied in different ways, often somewhat independently from one another. This distinctiveness was attached to procoagulant and regulatory MP that emerged relatively early from studies on hemostasis and vascular biology.[26,30,32–35] In contrast, MV were studied in the field of neurobiology and immunomodulation,[13,36,37] and EXSM have entered the scene largely in the course of studies on cellular differentiation,[19] endosomal trafficking and receptor recycling.[38–40] What adds to the confusion is that the term *exosome* has also been used to describe the intracellular ribonuclease complex, which is completely unrelated to cellular vesiculation.[41]

Nonetheless, MP/MV have emerged as a long known but newly appreciated mode of biological regulation, and they are increasingly implicated in various important contexts, especially during vascular pathology, immune responses, cellular differentiation, and other physiologically and pathologically significant events.[42–45] Their role is also increasingly recognized in cancer, including in such aspects of the disease as cancer coagulopathy,[26,30,34,46–50] activated stroma generation,[51] tumor growth,[22] establishment of the tumor stem cell niche,[49] invasion,[52] angiogenesis,[53–58] metastasis,[54,59–61] and immune responses/evasion.[12,23,58,62–66] Moreover, distinct molecular properties of MP present in the bloodstream and body fluids of cancer patients render them potentially useful as a novel and unique source of disease-related information.[23,62,67,68] Although this area still remains to be explored more fully (e.g., as a source of biomarkers), a considerable demand for such markers reflecting the natural complexity does seem to exist in terms of individualized patient care, precise molecular diagnosis, and monitoring the effects of targeted therapies.[18] The crucial questions that perhaps should be addressed before MV/MP are fully understood and utilized are those surrounding their biogenesis, biological roles, and their functional involvement in the pathogenesis of cancer and other diseases.

BIOGENESIS OF MICROPARTICLES

The properties of various MP are implicitly defined by their biogenesis. Cellular vesiculation has been appreciated and studied for several decades, but its exact mechanisms still remain surprisingly mysterious.[20] Indeed, the release of vesicular organelles was first described by Wolf in 1967, who noted formation of a particulate "dust" by activated blood platelets.[33] In a series of pioneering studies Johnstone observed that seemingly similar organelles (EXSM) were produced by differentiating reticulocytes, and were involved in the removal of "spent" transferrin receptors from the emerging red blood cells.[19] Formation of vesicles was also noticed in the case of cancer cells, in which their cargo was found to contain procoagulant mediators such as tissue factor (TF),[34] and, more recently, mucins

Table 1 Oncogenic Induction of Cellular Vesiculation

Oncogenic Pathway	Impact on Vesiculation	Reference
K-ras	Increased emission of TF-containing procoagulant microvesicles in colorectal cancer cells expressing mutant K-ras	Yu et al[74]
EGFRvIII	Increase in vesiculation and incorporation of EGFRvIII into microvesicles (oncosomes) in glioma cells transformed with this oncogene	Al-Nedawi et al[23]
p53	Increase in production of TF-containing microvesicles in colorectal cancer cells upon deletion of p53 gene	Yu et al[74]
p53	Increase in exosome production in cells, in which irradiation triggered p53 expression	Yu et al[75]
EGFR/AKT	Activation of the EGFR and AKT pathways stimulated vesiculation of prostate cancer cells	Di Vizio et al[28]

TF, tissue factor; EGFRvIII, epidermal growth factor receptor variant III; EGFR, epidermal growth factor receptor.

(MUC1).[29] These intriguing observations were subsequently extended to several other characteristics and linked to cancer-related biological events.[12,13,19] The association of MP production in the context of cancer is increasingly recognized[12,13,18,19,25,26,69] and raises numerous questions as to the implications, regulation, and molecular mechanisms of the underlying processes.

Biogenesis of MP/MV (vesiculation) is not unique to cancer and occurs during such processes as cellular differentiation, stress, activation, senescence,[70] stimulation with cytokines or shear force,[12] exposure to adenosine triphosphate (ATP),[71] apoptotic cell death,[27] changes in the microenvironment,[72] hypoxia,[73] and malignant transformation.[34] In the latter case, the action of mutant oncogenes, such as K-*ras*[74] and epidermal growth factor receptor (EGFR),[23] or its mutant, called EGFR variant III (EGFRvIII), appear to stimulate the release of MP (MV) in increased quantities (Table 1). Similarly, the activation[75] or loss[74] of certain tumor suppressors (e.g., p 53)[74] appears to impact cellular vesiculation. These examples likely capture only a small fragment of the oncogenic regulation of cellular vesiculation, either alone or in concert with various influences of the tumor microenvironment.[18] Interestingly, oncoproteins not only stimulate formation of MP/MV, but also become their cargo, a process described as formation of oncosomes.[22,23] Moreover, oncosomes may serve as a vehicle whereby oncogenic cargo may be transferred horizontally between cells, both transformed and nontransformed.[22,23]

Pathways mediating oncogene-dependent vesiculation are still somewhat obscure, but their elements are gradually coming to light. For instance, a recent elegant report by Di Vizio et al[28] suggested that MP production by prostate cancer cells could involve the activation of the Akt pathway and loss of the actin nucleating protein known as diaphanous-related formin 3 (DRF3/Dia2). Because DRF3 expression is lost during the development of metastatic disease, more aggressive prostate cancer cells may become increasingly prone to undergo vesiculation and to releasing active signaling proteins into their surroundings via oncosomes.[28] These processes may also involve Src activity.[71] Interestingly, Arf6, a small GTPase involved in cancer cell invasion, has been shown to promote shedding of proteolytic and proinvasive MV/MP from several types of transformed cells.[76] It is very likely that many additional molecular pathways could be involved as well. Their effects may resemble those involved in cancer-unrelated forms of vesiculation, including calcium fluxes, cortical actin reorganization, altered lipid metabolism, as well as many others.[35]

One important and poorly understood aspect of cellular vesiculation has to do with the assembly of the MP/MV cargo. Interestingly, studies on the proteomes of MP/MV reveal both similarities and differences vis-à-vis the protein expression profiles of the corresponding cells of origin.[62,77,78] The underlying molecular sorting mechanisms are probably quite diverse, and they are studied more in the case of certain types of MV/MP (e.g., EXSM)[38] than in other instances. It is believed that the cargo of MP may be related to the dynamics of plasma membrane domains, from which vesicles originate (e.g., formation of lipid rafts),[46] but what controls the content of the MV lumen is less understood. Clearly, the nature of the cell of origin is an important factor in these processes because membrane antigens of endothelial cells, platelets, and other cells are often also found on the surfaces of their corresponding MV/MP.[13,30] The functional state of cells (e.g., activation) also impacts the cargo of MP,[23] as does the specific type of MP being generated and their history post release. For instance, the composition of MP may change as a result of their possible fusion with other, even heterologous MP.[29] Also, secondary vesiculation events involving material from nonsecretory intracellular vesicles recruited to the plasma membrane[20] and plausible reemission of the material previously transferred from other cells may contribute to the MP/MV composition. Although the lineage markers are often used to establish the origin of MP,[25,30] the vesicle-associated protein, mRNA, and microRNA cargo may also differ considerably from that of the emitting cells.[62,77] In the case of PMP, the most abundant MP circulating in the peripheral blood, their

procoagulant cargo is concentrated up to two orders of magnitude as compared with the corresponding membranes of intact platelets.[79] This may suggest the existence of mechanisms that control a selective (rather than random) loading of the MP with certain types of cargo. It is plausible that in the case of cancer cells this loading process could be influenced by the underlying repertoire of cellular/genetic and signaling aberrations.[18]

PROCESSES UNDERLYING BIOLOGICAL HETEROGENEITY OF CELLULAR MICROPARTICLES

MP studied in different experimental settings have been distinguished from one another by several features. Their diverse origin, as well as molecular and morphological characteristics, led to the emergence of the elaborate nomenclature, including terms such as *tumor vesicles, cellular vesicles, shedding vesicles, microvesicles, microparticles, exosomes, ectosomes, enlargeosomes, promininosomes, prostasomes, epididimosomes, argosomes, archeosomes,* or *oncosomes.*[13,19,20,23,80] As mentioned earlier, some of these terms are used interchangeably with others or more exclusively, but not always, with the necessary consistency.[19] The concepts as to what exactly distinguishes different vesicles, and to what extent, also vary considerably. For instance, recent studies pointed to similarities in compositions between vesicles (EXSM) of different cellular origin including glioma cells. Such tumor-derived EXSM would mainly differ from their normal counterparts by harboring oncogenic receptors.[81] Other studies focus on diversity of MV/MP and highlight the distinct molecular makeup of their different subsets,[62,77] even if they emanate from the same cellular source.[82]

Indeed, heterogeneity among MP/MV is probably rather common. For instance, it is difficult to imagine that dramatic differences between cancer cells originating from different tissues would result in production of similar MV. Likewise, different genetic and microenvironmental influences affecting cancer cells would be expected to produce not only quantitative[23,74] but also qualitative differences[18,81] between MV being generated.[18] Moreover, different stimuli modulating the responses of normal cells associated with various cancers would likely change the profile of MP emanating from tumor stroma.[20] Some of these differences could be informative as to the pathogenesis of the underlying malignant process and thereby potentially useful for prognostic, predictive, or therapeutic purposes.[18] Perhaps most importantly, it is very unlikely that fundamentally different mechanisms of cellular vesiculation that could coincide in the same or different cells would lead to formation of uniform or otherwise similar vesicular structures, especially in changing disease settings. It is also possible that with progression of the same disease the underlying molecular processes and cellular diversification would produce changing patterns of MP/MV emission into the tissue and systemic circulation. In this regard, there are presently at least three different known major pathways that lead to emission of cellular vesicles, namely (1) through apoptotic cellular breakdown, leading to formation of apoptotic bodies, sometimes referred to as true microparticles;[83] (2) vesiculation associated with processes of plasma membrane blebbing and shedding; and (3) endocytosis-related formation of EXSM.[12,13,19,84] It is very likely that additional variations of these mechanisms may exist, leading to the emission of the heterogenous repertoire of MP.[82] The details of these distinct vesiculation processes are worthy of some commentary.

Apoptotic Microparticles

Formation of apoptotic MP represents a terminal consequence of membrane blebbing and cell fragmentation in the course of the classical programmed cell death.[85] An interesting consequence of this chain of events is that the resulting MP may continue to "posthumously" propagate some of the cellular material from their already nonexistent cells of origin, including their DNA fragments containing transforming genomic sequences.[86,87] It may be possible to recover these sequences from circulating apoptotic MP found in the blood of cancer patients.[88] Because formation of apoptotic MP effectively ends the existence of their cellular sources, the significance of these MP may be transient, unless the process of cell division, death, and apoptotic vesiculation occurs in a perpetual manner (e.g., in certain cancers).

Membrane Microvesicles

MP/MV are also generated by viable cells, often for extended periods of time, and especially upon their activation or transformation.[18] This occurs through the outward blebbing of the plasma membrane regions,[13,19,89] which gives rise to relatively large structures (>100 nm up to 1000 nm in diameter), often referred to as bona fide MP, MV,[13] shedding, or membrane (micro)-vesicles.[20] It has been suggested that such MP emanate preferentially from membrane lipid rafts, a notion consistent with their high content of raft-related proteins such as flotilin-1, TF,[46,74] certain cellular lineage markers,[35] and, in some cases, oncogenic growth factor receptors, such as EGFR.[23] Such MV may exhibit high levels of exposed phosphatidylserine (PS), integrins (e.g., β1), and metalloproteinases.[31] They may also express P-selectin glycoprotein ligand (PSGL)1 (e.g., in the case of macrophages) or contain cytokines and chemokines, such as interleukin (IL)1β, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)2, regulated

upon activation, normal T-cell expressed and secreted (RANTES), and other cargo.[31,58,90–92]

The mechanism of this mode of (exo/ecto)vesiculation entails a focal and temporary change in the state of the enzymatic apparatus that maintains the "tonic" asymmetry of plasma membrane phospholipids.[35] This energy-dependent mechanism ensures that phosphatidylcholine and sphingomyelin (SM) remain on the outer surface of the plasma membrane while PS and phosphatidylethanolamine are exposed intracellularly.[35] Such action may be executed by the set of calcium-responsive, lipid-translocating enzymes (flippases), the cluster of which required for vesiculation usually includes gelsolin (expressed mainly in platelets), aminophospholipid translocase, lipid scramblase, floppase, and calpain.[35,93] The release of cytosolic calcium causes profound changes in the state of these enzymes resulting in the inactivation of lipid translocase and activation of scramblase. This is followed by externalization of PS and changes in the membrane geometry, blebbing, and altered cytoskeletal interactions, all of which lead to the formation and eventual release of a "mature" MP/MV.[35]

The process of MP/MV generation may be activated by ATP and the related receptors (especially P2X7[90]), for example, upon the exposure of susceptible cells to the material released from their dying neighbors. This stimulation leads to mobilization of acidic sphingomyelinase (A-SMAse) and the resulting membrane phospholipid rearrangement. In the case of glial cells, this mechanism was found to be essential for microvesicular release of IL-1β This process may stimulate inflammatory responses, which are thought to help remove the dying cells (a source of the ATP).[71] Formation of microvesicles may occur more readily at plasma membrane sites containing a high abundance of cholesterol (e.g., membrane lipid rafts).[46] Unlike apoptotic bodies, such membrane raft–related MP do not contain cellular organelles, nuclear material, or DNA fragments.[94]

Physical events and mechanical forces leading to vesiculation are poorly understood. It is thought that the changes just mentioned in the phospholipid bilayer organization and the exposure of PS on the cell surface are linked with the formation of a localized physical "bend" of the plasma membrane. This is followed by a growing protrusion (blebbing),[95,96] eventually leading to MP maturation and release. Proteins have also been implicated in the formation of such membrane curvatures, acting in an either enzymatic or nonenzymatic manner.[97] Expansion of the membrane protrusions and disruption of the membrane-cytoskeletal connections is, at least in part, catalyzed by gelsolin and calpain, which contribute to the eventual shedding of the emerging MP/MV.[35] Moreover, these events could involve not only physical forces inherent to the emerging structures but also applied by contractile proteins that have been found in both mature MP/MV and in the "necks" or membrane protrusions before MP/MV shedding.[76] Because the emission of MP by activated cells leads to the loss of membrane material, the nonsecretory intracellular vesicles are thought to be mobilized to the cell surface. As they replenish the membrane lipids, they too could form MP and be shed to the pericellular microenvironment, thereby changing the repertoire of the emitted MP over time.[20] For various reasons the repertoire of circulating MP may also change at the systemic level, for example, as a function of gender,[98] circadian rhythm,[99] health status,[30] possibly cancer progression, and other factors.

Interestingly, aberrant microvesiculation is associated with certain hereditary conditions, such as Scott's syndrome, Castaman's syndrome, and possibly also Stormorken's syndrome.[30] These states are often manifested by bleeding propensities, suggesting that the impact of abnormal vesiculation on platelets is the most immediate (albeit not a sole) manifestation of the underlying defects.[35] Perhaps the best described in this regard is Scott's syndrome, where the impaired activity of the lipid scramblase leads to the inability of blood platelets to expose PS and to produce procoagulant MP.[35,100] Curiously, mice deficient in phospholipid scramblase 1 are hemostatically normal but instead develop defects in hematopoiesis.[101] This reveals a possible nonhemostatic role of MP[18,45] (or scramblase), raises questions as to the identity of MP-associated scramblase, and enforces the notion that mouse models may not always capture all the relevant aspects of human disease.[102] With this qualification, it is interesting to note that pharmacological blockade of PS, for example, by systemic injections of the annexin V analog (Diannexin), has shown some anticancer activity in mouse models,[18,22,103] possibly due to inhibition of microvesicular interactions[23] required for angiogenesis.

Exosomes

A fundamentally distinct form of vesiculation is involved in the biogenesis of EXSM.[12,13,18–20,39,49,65] Indeed, generation of these unique small MV occurs via the pathway of endocytosis rather than through a direct protrusion of the cellular plasma membrane.[38] In the case of EXSM, the initial budding of the plasma membrane occurs inward, at sites containing signaling receptor tyrosine kinases, whose recycling and regulation this mechanism controls.[38] The following evolution of vesicular structures is controlled by the endosomal sorting complex required for transport (ESCRT) system,[38,104] a set of multimolecular conglomerates responsible for the formation of the plasma membrane regions, in which the molecular cargo is processed for internalization. This entails tagging by ubiquitination and incorporation into inward budding regions of the plasma membrane.[38,105] The resulting caveolae, or coated pits, develop into

intracellular vesicles known as early endosomes. Cellular receptors present within endosomes may retain signaling activity but are ultimately destined for recycling or lysosomal degradation.[105]

During these processes early endosomes are transformed into more complex structures, known as multivesicular bodies (MVB), and late endosomes,[38] which evolve within the cytosol under the control of the ESCRT system. This leads to MVB fusion with lysosomes, where their cargo undergoes proteolytic destruction.[38] Alternatively, endosomal cargo may be recycled to the plasma membrane.[13,38] A third pathway associated with the endosomal system triggers a secondary inward membrane budding process within MVB, which results in formation of smaller intraluminal vesicles (ILV). ILV contain phospholipid capsule containing transmembrane receptors, which exhibit the outside-out orientation.[13,40,104] A fascinating process of lipid self-assembly has recently been described to explain the formation of ILV ("pre-exosomes").[104] In an elegant study Trajkovic et al demonstrated a spontaneous formation of small EXSM-like structures within synthetic larger lipid vesicles, simply through their enzymatic enrichment in ceramide. Addition of neutral sphingomyelinase (N-SMAse) in a cell-free system was sufficient in this case to trigger the inward vesiculation process similar to generation of ILV.[104] It is noteworthy that N-SMAse involved in this form of vesiculation is different from the A-SMAse involved in the generation of cellular membrane-derived MP/MV.[71]

It is thought that in the case of MVB that are destined for exocytosis the ESCRT processing for lysosomal destruction is aborted,[104] and the MVB are redirected to the plasma membrane, where they release their inner microvesicles (ILV; true EXSM[106]) into cellular surroundings.[13,104] Thus the unique biogenesis of EXSM renders them fundamentally different from the MP/MV originating directly at the plasma membrane.[13,82] Indeed, EXSM are much smaller in size (<50 to 100 nm) than MP/MV (100 to 1000 nm), and they contain different types of molecular cargo.[19,77] This includes a significant enrichment in heat shock proteins (HSP-70) and tetraspanins (CD63; Tspan8)[77,107] but lower content of exposed PS.[71] In some cases cells simultaneously produce several sizes (and types) of MV,[82] including EXSM, each type endowed with different cargo and biological properties.[108] In cancer, production of EXSM may be influenced by oncogenes and tumor suppressor genes.[74,75,109] In particular, the p53 gene product appears to regulate biogenesis of EXSM by upregulating TSAP6 protein in irradiated cancer cells.[75]

It is presently unclear how oncogenic transformation, tumor microenvironment, therapeutic agents, interactions with other cells, and other influences affect various pathways of cellular vesiculation in cancer. Oncogenic signals trigger formation of MP/MV by human glioma cells,[23] which are also known to produce ample amounts of EXSM.[62,81] In vivo, these pathways likely intersect with regulatory responses to hypoxia, inflammation, and cytotoxicity,[27] all of which may contribute to the generation of unique combinations of EXSM[109] and membrane MV,[23] and to changes in their content. Similarly, cell lineage definition, cancer type, cellular differentiation, and processes of epithelial to mesenchymal transition (EMT)[110] appear to influence patterns of cellular vesiculation, as does formation of the stem cell population[111] and the related intercellular interactions within the stem cell hierarchy.[45]

BIOLOGICAL ROLES OF THE VESICULATION PROCESS

There is no single or conclusive answer to the question why the mechanisms of MP/MV production have evolved in various organisms and what are their ultimate physiological roles.[12,13,20,26] This question is even more complex in the context of cancer, where the analyses of vesiculation have largely been correlative, and evidence in vivo as to the causative, or rate-limiting roles of MP/MV is relatively rare.[22]

It is not known whether hereditary defects in vesiculation, such as Scott's or Castaman's syndromes,[30] affect cancer progression and by what mechanism. However, inferences as to the role of vesiculation in cancer can be made from correlative studies in clinical and preclinical settings,[29,112] and from studies conducted in vitro. The latter suggest that production of MP/MV could serve as a rapid and efficient mode of removal (shedding), relocation, or transfer between the cells of certain molecular components. Such a mechanism could bypass the barriers associated with the relative insolubility of molecules and their complexes (e.g., of transmembrane receptors[23,113]) or inefficient secretion of certain ligands (e.g., those lacking signal peptides[31,71]). Vesiculation could also circumvent the inefficiencies of the lysosomal degradation system, as was described in the case of transferrin removal from reticulocytes.[19] It is possible that shedding of MP/MV containing oncogenic cargo (e.g., mutant EGFR)[18] may also be triggered, as a form of primordial cellular defense from the signaling "overload" that may be associated with overexpression of these highly active proteins.

MP are also a part of the antigen presentation and immunomodulation apparatus. In this capacity their shedding could mediate interactions between cancer cells and the immune system, where MP could serve "defensive" purposes. For instance, rapid removal of the complement attack complexes from opsonized cells[13] could potentially be activated in tumor cells, protecting them from complement-mediated lysis. Such cells could also use MV as a means to deploy various immunomodulating activities (e.g., cytokines or antigens), changing the

patterns of host responses.[36] In certain contexts, including oral, colorectal, and other cancers, MP/MV may also perform an "offensive" function, for instance, as carriers of Fas ligand. Contact with such MP/MV could induce apoptosis of innate and antigen-specific immune effector cells.[36,114–117]

In certain instances MP may also serve the purpose of "nonconventional" release and gradient formation by cytokines,[71] biological transmitters,[12] membrane-anchored receptors, adhesion molecules, enzymes, and signaling proteins.[12,13,23] This mode of release was implicated during formation of gradients of active morphogens involved in normal development.[118] It is noteworthy that similar molecular entities, such as wingless (Wnt)[119] or hedgehog (Hh),[120] have also been implicated in cancer.[45,121] Less clear is the role of mRNA and microRNA inclusion into various MP/MV. Again, the removal and/or intercellular transfer of these molecular species are the most compelling explanations of their role as cargo of MV/MP.[12,77]

INTERCELLULAR EXCHANGE OF MOLECULAR INFORMATION VIA MICROVESICLES

MV are known to transfer biologically active materials between cells.[35] However, the scope of biological consequences unleashed by this process are highly context specific and still poorly understood.[23,46,62,122] Most studies in this area revolve around such events as the activation of the coagulation system, inflammation, immune responses and neuronal communication,[12,20,33,34,123,124] and some pathologies including cancer.[12,18,34]

The significance of MV-mediated intercellular communication is usually inferred from the nature of molecules found within or on the surfaces of these structures.[53,58,125,126] The uniqueness of this communication stems from at least two properties, namely (1) the capacity to transfer multiple effectors at once, including molecules that are normally insoluble,[62] and (2) the inclusion of molecules in the active and/or otherwise intact state.[18] For instance, various soluble splice variants' cellular receptors, their fragments, and degradation products may be found in the intercellular space or blood but mainly in an inactive state and in the absence of their natural signaling partners and interactors. In contrast, many of the same entities (transmembrane receptors, membrane-bound ligands, signal peptide-deficient growth factors, cytoplasmatic and nuclear proteins, mRNA and microRNA) may exit cells as cargo of MV/MP, often intact and in their natural activation state (e.g., phosphorylated).[12] Their entry into another cell, therefore, may cause functional changes unachievable in any other manner.

It has recently come to light that in cancer, the intercellular, MV-mediated transfer processes may encompass cancer-specific molecules and activities, such as those associated with active oncoproteins,[127] as well as intact oncogenic RNA species.[62] However, other molecules of pathogenic significance could also be a part of this molecular exchange, whether emanating from cancer cells or their related host cell compartment.[12] Indeed, the available evidence supports the possibility of microvesicular "sharing" of TF and certain oncogenic receptors between cancer cells and endothelium,[22,128] a process that could clearly affect tumor angiogenesis. Similar transfers of transmembrane molecules could, at least in theory, include several other entities (e.g., VEGF receptors, Tie receptors, Notch receptors and their ligands, ephrins and Eph receptors, adhesion molecules, and integrins along with many others). It is intriguing to consider that vesiculation could in some instances extend the range, gradient, or change the signaling characteristics of the cell-associated (juxtacrine) ligand-receptor systems. Some intriguing clues to this effect have already begun to emerge from studies on the Notch system (Adrian Harris, personal communication).

Although direct studies in vivo on the microvesicular transfer are both difficult and scarce, several lines of more indirect evidence have recently emerged as to the possible biological significance of such a process. Thus microvesicular release/transfer has been implicated in the case of coagulation factors (e.g., TF[46,128]), chemokine receptors (CCR5),[113] adhesion molecules,[129] immunomodulators,[36] cell surface antigens,[125] intact RNA species,[12,77] and cancer-associated proteins (oncoproteins).[23,67,109] Similarly, the passage of carbonic anhydrase to lymphocytes by MV/MP[130] or transfer of phospholipids from red blood cells to their nucleated counterpart have recently been reported.[131]

Among these studies, a particularly instructive and, indeed, a seminal example was described by Mack et al.[113] These investigators demonstrated that a chemokine receptor (CCR5) is released from epithelial cells as cargo of MP, and those particles are subsequently taken up by mononuclear or endothelial cells. The resulting CCR5 transfer to these cells is highly consequential because it allows them to use CCR5 as an entry portal for the human immunodeficiency virus 1, thereby propagating the infection.[113] It is tempting to speculate that similar MP-mediated uptake mechanisms may apply to other receptors and may also sensitize cells to other viruses, prions, or to stimulation with alternative ligands. Such events could also provoke autocrine/intracrine responses to ligands that the recipient cells may already produce. The latter is exemplified by the activation of the VEGF/VEGFR2 pathway in endothelial cells stimulated with EGFR-bearing MV.[22]

Perhaps one of the most intriguing effects of MV is their ability to transmit differentiation regulating signals and effectively trigger the reprogramming of target cells. A fascinating example of such an effect is

described in the recent study by Ratajczak et al, who demonstrated the capacity of MV emanating from pluripotential embryonic stem (ES) cells to interact with hematopoietic progenitors, which are thereby induced to express genes related to pluripotentiality (Oct4, Nanog, Rex-1). In this case the MV recipient cells also acquired markers of early hematopoietic differentiation (Scl, HoxB4), along with biological and growth responses attributable, at least in part, to the uptake of the ES cell–related mRNA cargo.[45] In another elegant study, hedgehog (Hh) proteins were found to be emitted in the cargo of MV produced by stimulated T cells, and their uptake led to the reprogramming of K562 erythroleukemic cells, or primary CD34 + cells toward the megakaryocytic linage.[121] MV were also implicated in morphogenic and developmental events in nonmammalian systems in vivo,[118] and there is no reason to exclude such interactions in higher organisms.

The emerging evidence suggests that emission of MV and EXSM may participate in formation of the growth-supporting niches for stem cells in various physiological or pathological settings.[49,55,60,132] Such MV exchanges may occur between vascular, inflammatory, and transformed cells, and influence multicellular effects associated with cancer initiation, progression, angiogenesis, and metastasis.[12,18,31] The potential of membrane MV and EXSM in this regard is underscored by several recent comprehensive studies on their molecular content (proteome, transcriptome, and profile of microRNA). Such profiling studies have been performed in several cell systems, including mast cells,[77] fibroblasts,[78] epithelial cells,[133,134] endothelium,[27,135] and different types of cancer cells.[62,134,136–138] For instance, MV derived from human glioma cells were demonstrated to contain a wide spectrum of proteins, cytokines, chemokines, and ~4700 unique transcripts that were not detected (or differed in abundance) in the transcriptome of the corresponding tumor cells.[62] Interestingly, this analysis was performed on tissue isolates, which normally contain different cellular subsets. Consequently, the heterogenous cellular sources could result in heterogenous composition of MV/MP and thereby increase the diversity of microvesicular mRNA, microRNA, and proteins,[62] very much as would be expected to occur in vivo.

Although most of the MV/MP studies are performed in cell culture systems, there is a growing body of evidence supporting the occurrence of microvesicular transfer in vivo.[23,139,140] For instance, MP/MV containing TF are readily detected in the blood of experimental animals and cancer patients,[26,29,139] and EGFRvIII containing oncosomes has been detected in blood and in inoculates of cancer cells in mice.[23] In such settings, MP/MV are thought to be short lived (up to 20 to 60 minutes in the circulation[139,140]), likely due to their rapid uptake by target cells or through other forms of bioelimination (possibly via interaction of their surface PS with the phagocytic system). However, the biological consequences of MV uptake may be relatively long lasting (days).[23] This combination of potent impact and short half-life may endow a microvesicular mode of signal delivery with unique characteristics.

The mechanisms of MV/MP uptake by various cells are a subject of ongoing studies. In some instances the evidence points to specific recognition mechanisms, for example, mediated by molecules expressed on the surfaces of MV/MP and those of recipient cells. For instance, the uptake of procoagulant MP by platelets may occur in a manner that depends on their expression of P-selectin, which recognizes the PSGL-1 on the membranes of MP.[129] In other instances, MV may use their exposed PS to bind to the corresponding cellular PS receptors (PSRs). Such PSRs are often expressed by macrophages, and they participate in the phagocytic recognition of apoptotic cells, which also have a compromised lipid asymmetry and expose PS on their outer surfaces. Interestingly, PS-positive MP may bind to viable cells as well and "falsely" tag them for macrophage recognition.[131] In contrast, MP may interact with PSRs on viable cells in a manner that does not necessarily provoke phagocytosis but instead leads to a merger between the interacting plasma membranes. PS moieties present on the surface of microvesicles can be blocked using annexin V or its derivatives (Diannexin), an effect that attenuates the MV uptake by target cells and prevents the exchange of their cargo.[23,46] Although EXSM are often regarded as exposing relatively low levels of PS, PSRs have also been implicated in their cellular uptake.[141] Several PSRs have recently been described, including Tim1, Tim4, stabilin 2, or BAI1,[141,142] at least some of which may be involved in the cellular uptake of PS-positive MP in various settings.[141] It has also been proposed that by interacting with Tim1/4 receptors on two adjacent cells, microvesicles could create intercellular bridges and thereby promote additional intercellular interactions.[141] Interestingly, one of the PSRs (BAI1) is also known as a potent endogenous angiogenesis inhibitor. BAI1 was originally isolated from brain tissue and found to be regulated by the levels of p53.[143] Whether the role of this receptor in angiogenesis is related to its interactions with PS-positive MP remains both highly intriguing and relatively unexplored. It is thought provoking that both cellular vesiculation[23,74,75] and some of the mechanisms of MV/MP uptake (e.g., BAI1) could be controlled by oncogenic pathways. A corollary to this point could be that genes involved in malignant transformation could alter the level of intercellular communication via MV/MP.

It is not always clear what type of membrane fusion mechanisms are used by MP/MV interacting with surfaces of their target cells. For instance, subsets of membrane-derived MP endowed with low PS content[122] or

cellular EXSM may employ alternative modes of interactions with their recipients. In the case of some vesicles (e.g., synaptic vesicles), their contact with the plasma membrane may occur in a protein-assisted manner, which is thought to lead to direct interactions between juxtaposed lipid surfaces.[144] Experiments involving differential fluorescent labeling of membrane lipids revealed changes in the emission wavelength upon MP uptake.[46] This is indicative of the mixing process between MP-related and cell-associated membrane phospholipids.[46] Similar conclusions can be drawn from the lengthy retention on the surfaces of target cells of MP-derived fluorescent dyes, catalytic activities (e.g., procoagulant TF activity), or transmembrane receptors (e.g., TF, EGFR; CCR5). Other mechanisms of MP/MV uptake have also been suggested, including intracellular penetration of intact vesicles. This is inferred from detection of such vesicles and their cargo within the cytoplasm of the acceptor/target cell (e.g., using immunological or electron microscopy methods).[6,62] The consequences of these different modes of MV/MP–cellular contact are poorly understood.

MICROPARTICLES AS REGULATORS OF THE COAGULATION SYSTEM IN CANCER

Historically, the prominent biological role of MP/MV was first detected,[32–34] and subsequently has been long studied, in the context of the coagulation system.[26,30,122] This area has been covered extensively by several excellent recent reviews,[25,26,30,122,145–147] and therefore we concentrate only on selected key questions. In this regard, the long-standing puzzle is related to the exact pathomechanism of the systemic coagulopathy in patients with localized and especially advanced cancers who also may exhibit high levels of circulating MP/MV.[148–151]

In spite of its long history,[152] the linkage between cancer and abnormal coagulation is still surrounded by several unanswered questions, including (1) Are the mechanisms of cancer-related thrombosis cancer specific and to what extent? (2) Is this specificity (if any) linked to properties of cancer cells and in what ways? (3) What mediates systemic changes associated with events (tumors) that may occur locally? (4) What are the implications of cancer-dependent molecular changes for thrombosis-related patient survival? and (5) What are the implications of hemostatic perturbations for anticancer therapy? Inclusion of MP in studies related to these questions has recently led to several useful clues.

Cancer patients exhibit heightened levels of circulating procoagulant MP that correlate with the risk of thrombosis.[26,29,48,112,146,153] In some instances, such procoagulant MP may emanate from platelets or inflammatory cells, or be produced by fusion between vesicles of different origin.[29] However, there is an increasing appreciation for the notion that cancer cells themselves may be a source of procoagulant MP. This is suggested by the nature of the procoagulant activity associated with such MP, which appears to reside in their content of PS, TF, and MUC1.[26,29] The latter two types of cargo are often upregulated by cancer cells.[29,149,151] Moreover, the levels of circulating MP are often diminished after the surgical tumor removal.[146] In experimental studies, procoagulant MP were found to home to sites of ongoing thrombosis. More specifically, in mice harboring tumors engineered to emit fluorescent MP (tagged with enhanced green fluorescent protein), the experimental bleeding times were generally shortened, and the fluorescent signal associated with procoagulant (tumor-derived) MP accumulated in emerging clots.[139] This suggests that cancer cells may be a major source of procoagulant TF-containing MP circulating in blood and that such MP may exert systemic vascular effects,[139] at least in part as a function of malignant transformation.[74,154]

Indeed, oncogenic transformation emerges as a major trigger of both cancer coagulopathy and cellular vesiculation.[18,155] Mutant K-ras, EGFR, and p53[23,74] upregulate TF expression by cancer cells and have been shown to directly trigger the emission of TF-containing MP.[74,110] Certain other cancer-related processes such as formation of the stem cell (CSC) compartment and EMT may also lead to both TF overexpression and the release of TF-bearing MP.[110,156] Interestingly, cancer treatment with oncogene-directed (targeted) agents appears to diminish the extent of thrombosis in patients. This has been reported for acute promyelocytic leukemia in the course of treatment with all-trans retinoid acid, which inhibits the activity of the RARα – PML oncogene.[157] Similarly, in gastrointestinal stromal tumors (GIST), treatment with imatinib mesylate (Gleevec) was found to reduce coagulopathy.[158] The latter finding suggests that the activity of the c-Kit oncogene, which drives pathogenesis of GIST, may be involved in the deregulation of coagulation effectors in this disease, conceivably including TF and/or release of procoagulant MP.

MP are increasingly well established as carriers of procoagulant activity throughout the systemic circulation.[159] As mentioned earlier, the levels of circulating procoagulant MP correlate with the risk of cancer-related thrombosis.[25,26,29,30,46,112,160] In contrast, deficient vesiculation correlates with bleeding propensities in patients with Scott's syndrome.[35] It is not known whether such patients develop cancer and what the biological and procoagulant characteristics of the disease in such settings would be.[18] Further suggestion that MP may be central to the coagulation process come from experiments demonstrating the transfer of coagulation regulators between cells, a process found to contribute to the assembly of active procoagulant complexes on cellular surfaces.[12,26,30,122] Effects of this nature may result in the amplification of TF activity,[25,26,30,34] and MP-mediated

accumulation of this receptor has been documented for endothelial cells,[47,128] platelets,[46] and with growing thrombi.[139]

The role of MP in cancer coagulopathy deserves a few additional comments. Although cancer cells may make a direct contribution to the pool of circulating procoagulant MP (as previously outlined), their influence may also be indirect, for example, mediated through release of soluble mediators that mobilize and activate other cells (platelets, phagocytes, endothelial cells, or fibroblasts).[128,161,162] Given the emerging role of MP in driving cancer-related coagulopathy, therapeutic strategies targeting MP may represent an interesting and relatively unexplored option in this particular setting. In this regard, certain novel antithrombotic agents directed at PS, notably Diannexin,[163] could potentially be of interest (Meehan et al, unpublished data,[22]). Furthermore, although the most attention has been focused on cancer-related procoagulant MP, these vesicles could also harbor or induce anticoagulant and fibrinolytic proteins, including tissue factor pathway inhibitor (TFPI),[26] activated protein C and its receptor, thrombomodulin,[122] or urokinase.[164] Although procoagulant (and anticoagulant) cancer-related MP have a potential to modulate not only coagulopathy but also tumor angiogenesis, progression, and metastasis, few studies have examined these latter issues in detail.

THE INVOLVEMENT OF MICROPARTICLES IN TUMOR ANGIOGENESIS

Membrane vesicles and EXSM have been implicated in various aspects of vascular regulation, including the tumor-vascular interface.[22,53–56,92,165–170] Indeed, the involvement of the vascular system in malignancy is well established and multifaceted.[171] This entails not only the supply of oxygen, growth factors, metabolites, and hormones but also regulatory (angiocrine) functions and the role of a conduit for metastasis.[172] The extent to which these processes are clinically relevant is reflected by the recent approval of at least four antiangiogenic agents (many more remaining in the pipeline), as the mainstay or as adjunctive anticancer therapy.[172,173] This list currently includes bevacizumab, sunitinib, sorafenib, and pazopanib, all of which are directed, at least in part, against VEGF and its endothelial receptors (VEGFR1–3). Both the angiogenesis process itself[174] and antiangiogenic therapy[175] are also associated with perturbations within the coagulation system, which may involve processes mediated by procoagulant MP described earlier. There are, however, emerging suggestions as to coagulation-independent activities of various cellular vesicles (including membrane MV and EXSM) emanating from cancer cells and stroma in tumor angiogenesis.[18,53]

Tumor neovascularization is triggered and maintained by a complex network of interactions between various subsets of cells.[176,177] This includes heterogenous tumor cell subpopulations (cancer stem cells/tumor initiating cells and their progeny), stromal fibroblasts, resident endothelial cells, endothelial progenitor cells, inflammatory cells, and their bone marrow–derived precursors and platelets, all of which contribute different activities to the emerging tumor microcirculation.[176,178,179] The onset of this process can be traced to the combined and/or sequential change in expression of angiogenesis-regulating genes (stimulators, inhibitors, and modulators), which is triggered by activated oncogenic pathways, hypoxia, and inflammatory mediators.[180] These events are traditionally described in (uni)molecular terms. Thus growth factors such as VEGF, angiopoietins (Ang1/2), ephrins, and delta-like 4 (Dll4) act on their respective receptors (VEGFR1–3, Tie2, Eph, Notch),[172,177] and in concert with adhesion molecules, integrins, and their extracellular matrix they are thought to orchestrate the neovascular expansion.[177–180] Moreover, these interactions activate several pathways of blood vessel formation,[176,177,181,182] in which endothelial cells, pericytes, bone marrow derived (regulatory) cells,[179] endothelial progenitor cells (EPCs), and platelets[183–185] play relatively well-defined and complementary roles.[26,177,178] In this regard, one of the best described is the process of vascular sprouting. In this case the activated endothelial cells of the vessel wall (phalanx cells) deploy cohorts of endothelial cells (sprouts). Sprouts are composed of columns of growing and migrating endothelial cells (stalk cells) moving in the direction defined by the angiogenic gradient (e.g., concentration of VEGF), which is detected by specialized leading cells (tip cells). Tip cells are equipped with high levels of VEGF and PDGF receptors, as well as other characteristics such as high levels of the Notch ligand, Dll4.[179,186] Angiogenesis is modulated by the influences of other regulatory systems. For instance, the coagulation system impacts the angiogenic process through release of regulators from platelets but also through effects afforded by the fibrin clot and the signaling input of TF, factor Xa, thrombin, and protease-activated receptors.[74,183,187–190] Other pathways may also modulate the outcome of vascular growth, and the details of the related events have been summarized elsewhere.[177,179] Operationally then, tumor neovascularization entails the expansion of the preexisting microvascular network (angiogenesis), recruitment of EPCs to the sites of blood vessel formation (vasculogenesis), remodeling and regression of the emerging structures,[177] as well as cooption and invasion of normal vessels by cancer cells.[176,178,191]

There is presently no conclusive evidence that angiogenesis cannot proceed in the absence of vesiculation. This can be inferred from the disparity between the dramatic phenotypic consequences (lethality) that accompany deficiencies in expression of the so-called

professional angiogenic pathways (e.g., VEGF/VEGFR) and much milder consequences of disrupted cellular vesiculation. The latter is observed in patients with Scott's syndrome, where vascular development is essentially spared.[35] However, under more specific circumstances, cellular vesiculation (e.g., occurring in endothelial cells, cancer cells, and platelets) may play an important role in the stimulation and modulation of tumor angiogenesis.

Indeed, as mentioned earlier, tumor- and platelet-derived MV are a rich source of angiogenic growth factors (VEGF, FGF),[31,58] proinflammatory cytokines (IL1β[71], proteases (MT1-MMP)[54] and their inducers (CD147/EMMPRIN),[192] all of which could contribute to the proangiogenic intratumoral milieu, either directly or indirectly.[12,31] This could occur through several processes, including (1) intercellular transfer or proangiogenic cargo, (2) pericellular release of proangiogenic content of MV, or by (3) induction of proangiogenic gene expression upon contact between vascular cells and MV.[22,26,53,54,193,194]

Thus proangiogenic effects could be induced though the endothelial uptake of molecular cargo containing activating molecules. For example, endothelial progenitor cells emit MV containing mRNA, which can be transferred to resident endothelial cells causing their angiogenic activation.[55] Endothelial cells can also respond to transfer of mRNA from tumor cells[62] or to the uptake of MV containing active EGFR oncoproteins.[22] In the latter case, endothelial cells become positive for EGFR, both in vivo and in vitro, and they initiate production of endogenous/autocrine angiogenic activity (VEGF). This leads to activation of VEGFR2, an effect resistant to addition of VEGF-neutralizing antibodies (Avastin) but obliterated by intracellularly acting VEGFR2 kinase inhibitors (SU5416). In this setting, the blockade of MV-associated EGFR using a pan-Erb inhibitor (CI-1033) and blockade of PS (using Diannexin) abrogate the proangiogenic effects. Notably, Diannexin treatment in vivo produces inhibition of EGFR-driven tumor growth and leads to a reduction in microvascular density (MVD).[22]

It is of interest that a vesicular transfer of EGFR into cells that normally express this receptor at low levels may sensitize these cells to EGFR ligands (e.g., EGF). In the case of endothelial cells, this may result in an acquisition of proangiogenic activity by ligands that may normally stimulate this process weakly or not at all. Similarly, endothelial cells exposed to MV containing TF[128] (and possibly PARs) could become more susceptible to stimulation by the signaling effectors of the coagulation system, including factor VIIa and thrombin, both already implicated in angiogenesis regulation.[161,195] Indeed, this could be one way by which an increase in levels of procoagulant MV/MP circulating in the blood of cancer patients could promote tumor angiogenesis and progression. Although these possibilities are intriguing, the extent to which reprogramming of endothelial cells may occur via MV-mediated transfer of "heterotypic" receptors (Fig. 1) remains to be investigated in more detail.

Intercellular transfer of MV may also operate between cancer cells, leading to amplification of their angiogenic phenotype. In this regard, it has been proposed that in spite of their proangiogenic effects, oncogenic mutations in individual cells may not be able to trigger an overt onset of vascular growth. This is because such cancer cells would be surrounded with non/anti-angiogenic stromal cells or poorly angiogenic masses of indolent cancer cells, whose inhibitory effects may be difficult to overcome.[127] However, angiogenic switch in cancer would occur more readily if a coordinated proangiogenic change could be induced in multiple cells.[127] One way of achieving such an effect could be through the intercellular exchange of MV.[23] Indeed, glioblastoma cells expressing mutant EGFRvIII oncogene emit MV, which can be taken up by indolent tumor cells, followed by this receptor activating (ectopically) its downstream targets, such as VEGF.[23] In this manner a relatively smaller number of cells harboring the mutant EGFRvIII gene may affect a larger population of cells, in which the microvesicular uptake of the respective oncoprotein may induce a "collective" angiogenic phenotype.[18] Again, direct evidence for this scenario is to be established, but it is striking that expression of EGFRvIII in human glioblastoma occurs almost always in a minority of tumor cells while affecting the disease as a whole.[196]

As mentioned earlier, vesicles emanating from tumor and stromal cells may serve as an alternative mechanism for the release of angiogenic factors, cytokines, or proangiogenic enzymes that are cell associated, lack signal peptides, or are unstable in the extracellular environment. For instance, in spite of the inefficient standard secretion mechanisms, IL-1βis released from ATP-stimulated glial cells via membrane MV.[71] Similarly puzzling is the long-standing paradox of the high paracrine and proangiogenic activity of fibroblast growth factor (FGF)2, which lacks the signal peptide. Again, this could be explained by evidence that this growth factor can be released in a form of MV.[197,198] In spite of the efficient molecular release of VEGF, this factor is also found in the cargo of tumor-related MV and is liberated upon disruption of the MV membranes.[58] It is possible that this form of VEGF release protects this and other factors from proteolytic degradation. As mentioned earlier, MV may be recognized by target cells (whether transformed, inflammatory, or endothelial) via a mechanism involving PS and PSRs.[141] One such PRS, known as BAI1, is a known inhibitor of angiogenesis, which raises questions as to the role of microvesicular uptake in angiogenesis regulation.[12,22,31]

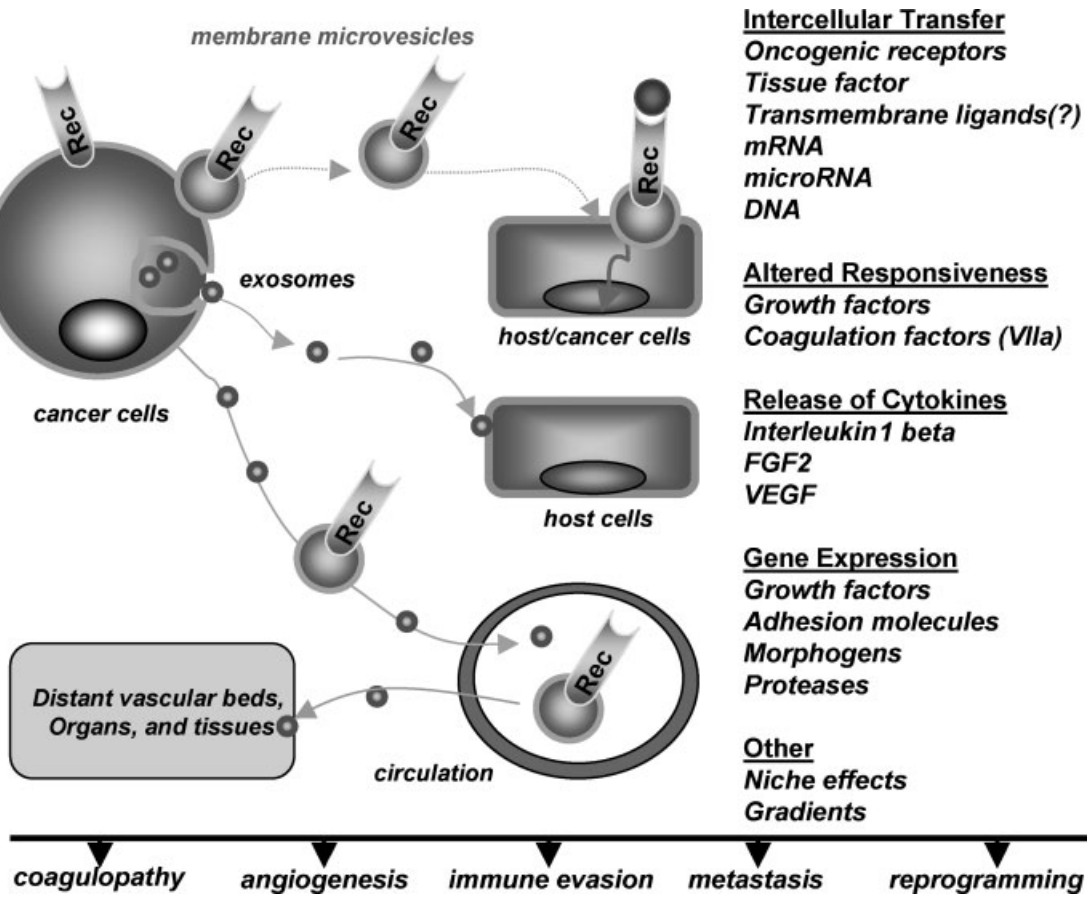

Figure 1 Microparticle-mediated biological responses in cancer. Both plasma membrane-derived microvesicles and intracellularly formed microvesicles (exosomes) are emitted by cancer cells and stroma and contribute to integrated cellular responses. The nature of this influence may involve transfer of microparticle cargo or stimulatory effects on endogenous production of various effectors. Oncoproteins and regulatory receptors (e.g., epidermal growth factor receptor) stimulate vesiculation and become a cargo of cancer-related microvesicles, which transfer them to other cells. Processes affected by microparticle exchange include coagulopathy, angiogenesis, metastasis, and many others. FGF2, fibroblast growth factor receptor 2; VEGF, vascular endothelial growth factor.

The induction of angiogenic activities in various cells (e.g., cancer cells or endothelium) may occur by the novo gene expression or mRNA transfer upon contact with MV and EXSM. Thus Janowska-Wieczorek et al reported a multifactorial change in the angiogenic phenotype of human lung cancer cells in vitro upon their exposure to PMP.[54] In this instance, the target cells were found to take up the platelet antigen CD41 while at the same time exhibiting increased MAPK phosphorylation and expression of transcripts for MMP9, VEGF, IL8, and hepatocyte growth factor.[54] Although the specific mechanism of this induction is presently unclear, another study explored the impact of EXSM bearing Tspan8/D6.1A/CO-029 tetraspanin on the activation of endothelial cells during tumor-driven angiogenesis.[44,53] This proangiogenic effect directly depended on the Tspa8 tetraspanin, as indicated by experiments with specific neutralizing antibodies. The proangiogenic effects of these EXSM resulted in VEGR-dependent and independent deregulation of several genes, including chemokines (CXCL5, MIF), their receptors (CCR1), and VEGFR2.[44] Microvesicles could also stimulate angiogenesis through a mechanism dependent on their lipid membranes (SM)[166] and through other mechanisms leading to deregulation of cytokines in endothelial cells.[57] Overall, these observations raise the possibility that MV/MP-directed agents could have a role in antiangiogenic therapy.[18]

THE ROLE OF MICROPARTICLES IN CANCER PROGRESSION AND METASTASIS

As mentioned earlier, MV/MP production can be triggered by oncogenic receptors (e.g., EGFRvIII), which subsequently become incorporated into the MV cargo and exit the cell of origin.[23] In this form, oncogenic EGFR and EGFRvIII were shown to be taken up by indolent cancer cells and normal cells.[22,23] Such horizontal transfer of the MV content into various "acceptor"

cells profoundly alters their properties, beyond the previously mentioned stimulation of angiogenesis.[22] In particular, microvesicular transfer of the active EGFRvIII oncogene from aggressive glioma cells to their indolent counterparts stimulated activation of MAPK and AKT pathways in these cells and altered their expression of genes regulating cell survival (BclxL) and proliferation (p27). This treatment also stimulated formation of three-dimensional colonies in semisolid medium (a hallmark of malignant transformation).[23] Importantly, the treatment of MV containing oncogenic EGFRvIII with the irreversible pan-Erb kinase inhibitor diminished these effects, as did cloaking their PS residues with annexin V[23] or Diannexin.[22] Similarly, microvesicles collected from fresh isolates of human glioblastoma were found to stimulate proliferation of the U87 malignant glioma cells, and a similar material was also shown to contain mRNA encoding the mutant EGFR (EGFRvIII).[62] EGFRvIII and EGFR were also found in tumor cell–derived EXSM.[99,109]

Several studies reported the impact of MV/MP on metastasis.[28,54,60,61] Thus PMV/MP contribute to the metastatic phenotype of cancer cells.[54,193] Tumor cells themselves may emit PS-containing MV that facilitated experimental dissemination of melanoma cells in mice.[61] Exosomes were found to cooperate with CD44v6 in conditioning premetastatic niches for colonization by pancreatic cancer cells.[60] Prometastatic proteins are released as a cargo of EXSM produced by epithelial cancer cells under hypoxia, along with alix and tertraspanins (CD9 and CD88).[73] Increased production of TF-containing MP/MV correlates with induction of the proinvasive phenotype (EMT) in EGFR-driven cancer cells.[110] In the course of human prostate cancer progression toward metastatic disease, the loss of DRF3 gene expression correlates with an increased vesiculation.[28] These and other studies that have been reviewed recently[12,26] point to the systemic, proinvasive, and vascular effects of MP/MV, all of which may play a role at various stages of tumor dissemination. Although these results are intriguing, the functional requirement for vesiculation has not been conclusively established in the course of metastatic disease in clinical settings. Nonetheless, it is of interest whether agents blocking MP/MV production, uptake, or activity could be effective in treating disseminated cancer, either in an adjuvant setting or in the course of overt metastatic disease.

ANTITHETICAL EFFECTS OF MICROPARTICLES AND EXOSOMES IN CANCER

Although MP are often discussed as carriers of stimulating, or pathogenetic influences (e.g., in cancer progression, angiogenesis, and coagulopathy), it is possible that under certain circumstances they may also exert the opposite (inhibitory, cancer-suppressive) functions. For instance, it is of interest that EXSM contain a rich repertoire of micro RNA (miRs),[77,199] including in the context of glioblastoma[62] and possibly in other tumors. Indeed, miRs may exert either oncogenic or tumor-suppressive influences, depending on the nature of genes whose expression they regulate.[199,200] Therefore, the action (transfer) of EXSM containing various miR species on cancer cells may be either stimulatory[201] or inhibitory. The latter may apply to EXSM emanating from normal cells. Similarly, normal cells (stromal fibroblasts, inflammatory cells) may emit MP that contain growth-suppressing and anti-inflammatory proteins, for example, transforming growth factor (TGF)β1,[202] products of tumor suppressor genes (e.g., maspin),[75,203] phosphatases,[204,205] immunomodulators,[90] and entities endowed with antiproteolytic (e.g., TIMP1),[206] anticoagulant (e.g., EPCR or TFPI),[26,122,207] or antiangiogenic activities (e.g., platelet factor 4).[75,208] It should be mentioned, however, that with the emergence of the activated stroma[209] and the related proangiogenic field effects orchestrated by cancer cells,[210] the impact of normal cells may evolve and become tumor promoting over time. Similarly, the profiles of MP/MV and EXSM could evolve accordingly. Like the many other questions surrounding MP/MV biology, these possibilities require further studies.

CIRCULATING MICROVESICLES AS BIOMARKERS IN MALIGNANCY

MP released into blood, urine, and body fluids offers a unique opportunity to access, noninvasively, the biological information directly related (pathognomonic) to cancer and stromal cells.[23] In addition, during surgical tumor tissue collection, or at biopsy, the regional differences in tumor cell properties may lead to sampling errors.[211] In contrast, circulating MV/MP are readily accessible and could be subjected to a molecular analysis of varying depth and focus. In this regard, xenotransplants of human glioma shed MV containing oncogenic EGFRvIII, which can be readily detected in the circulating blood of tumor-bearing mice.[23] EGFRvIII protein and mRNA[62] could also be retrieved from MV/MP, or EXSM circulating in the blood of patients with malignant glioma.[62,81] Similarly, the HER-2 oncoprotein could be detected in MV/MP isolated from culture media incubated with breast cancer cells.[67]

Indeed, the MV/MP-based technology could potentially yield useful prognostic and predictive insights. In this regard, selective or multiplexed assessment of oncogenic targets present in the cargo of blood-borne MV/MP, their status, splicing, posttranslational processing, and/or phosphorylation could be incorporated into protocols designed to monitor the effects of antitumor (Herceptin, Tarceva, Erbitux) and antiangiogenic

(Avastin, Sutent, Nexavar) therapies.[23,62,67] This could also potentially include testing for oncogenic mutations,[62] as well as mutations that may confer sensitivity or resistance to pharmacological inhibitors.[212]

CONCLUSIONS

Studies on tumor-related membrane MV and EXSM have recently entered a phase of rapid progress.[12,23,62,125] Cellular vesicles represent a fascinating and potentially useful reservoir (sanctuary) of biological information as to cancer-related processes such as oncogenic transformation, risk of thrombosis, angiogenesis, and other aspects. Their functional role still needs to be understood more fully, but the usefulness of various MP as disease biomarkers deserves immediate and extensive exploration.

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