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REVIEW

Microvesicles in Health and Disease

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Abstract Microvesicles (or MVs) are plasma membrane-derived vesicles released from most eukaryotic cells constitutively during early apoptosis or at higher levels after chemical or physical stress conditions. This review looks at some of the functions of MVs in terms of intercellular communication and ensuant signal transduction, including the transport of proteins (unconventional protein export) as well as of mRNA and microRNA. MVs also have roles in membrane repair, the removal of misfolded proteins, and in the control of apoptosis. We also discuss the role MVs have been shown to have in invasive growth and metastasis as well as in hypoxia in tumours and cerebral ischaemia. The association of MVs in infectious and autoimmune disease is also summarised together with their possible use as therapeutic agents.

Keywords Microvesicles · Intercellular transport · Cancer · Autoimmune disease · Stroke · Infectious disease

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Microvesicles

Microvesiculation is a ubiquitous cellular mechanism, which occurs as a result of exocytosis or direct release of vesicles from the cell surface membrane (Hugel et al. 2005). Cells are challenged continually by various chemical or physical stress conditions, which lead to the release of submicron microvesicles (MVs) ranging between 0.1 and 1 μm and those released through exocytosis, termed exosomes (50–100 nm; Fig. 1; Ratajczak et al. 2006b). MVs released from healthy viable cells are smaller in size than apoptotic bodies or blebs, derived from damaged cells which contain damaged DNA. Released MVs comprising membrane and cytoplasmic constituents contain numerous proteins and lipids characteristic of their parental cell.

Research into the nature of MVs has identified various functions including that of intercellular communication (Scanu et al. 2008), control of apoptosis and apoptotic response to stress (Sarkar et al. 2009), immunological roles (Nolan et al. 2008), procoagulant roles (Kim et al. 2005), cellular repair/housekeeping (Tomas et al. 2006), the export of proteins (del Conde et al. 2005), RNAs (Deregibus et al. 2007), peptide hormones (Hugel et al. 2005) and possibly cellular waste products such as misfolded proteins (Putz et al. 2008), cytotoxic agents and cell metabolic waste (Hugel et al. 2005). The release of MVs from many different cell types has led to the plethora of terms used to describe MVs in a host of reports. For example, MVs derived from activated blood platelets and other cell types such as monocytes are often referred to as microparticles (George et al. 1982). Membrane MVs released by activated human polymorphonuclear neutrophils are described in the literature as ectosomes (Hess et al. 1999). For the purposes of this review, the term MVs will be defined to exclude exosomes. MVs and exosomes have different pathways of

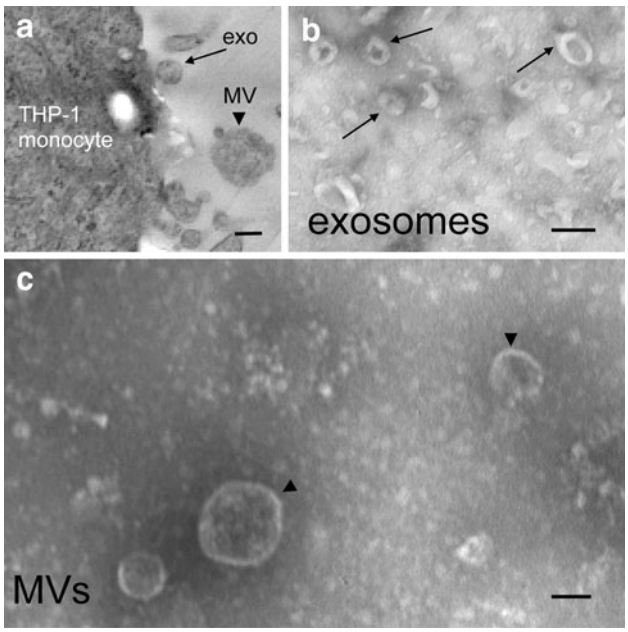

Fig. 1 Electron micrographs of microvesicles (MVs). Transmission electron microscopy of **a** exosomes and MVs released from a THP-1 monocyte cell and of purified exosomes (**b**) isolated from THP-1 monocytes (160,000*g* for 16 h) and of purified MVs (**c**) isolated by differential centrifugation at 25,000*g* for 90 min. *The bar* in all three panels is 100 nm, *arrowheads* indicating MVs and *arrows* indicating exosomes. *MVs* microvesicles

biogenesis and harbour different proteins, but few comparative studies of their respective roles are available. Although the main emphasis of this review is on MVs, this will not be to the exclusion of exosomes.

MVs are released into the extracellular microenvironment (in vivo) or into culture supernatants (in vitro) during growth or at apoptosis or upon activation. However, depending on their cellular origin, they may differ in size, biochemical composition and biological effects. We found these characteristics to vary according to whether MVs are released constitutively or upon stimulation (Stratton et al., submitted to Biochem J). During the process of MV release, normal cell phospholipid asymmetry is lost and this leads to increased exposure of phosphatidylserine (PS) on the outer leaflet of the MV membrane. This phenomenon is detected through binding assays with annexin V, a widely accepted method of identifying and quantifying MVs by flow cytometry. Studies based on MVs derived from platelets (Scholz et al. 2002), monocytes (Satta et al. 1994) and endothelial cells (Sadallah et al. 2008) have been well documented, but more recently, extensive studies have been carried out on neutrophils (Gasser et al. 2003), erythrocytes (Sadallah et al. 2008), granulocytes (Pap et al. 2009) and adipocytes (Aoki et al. 2010).

MVs were first reported in 1949 when it was recognised that a precipitable factor that accelerates thrombin generation was present in platelet-free plasma (Chargaff and West 1946). However, it took a further 20 years to isolate lipid-rich platelet cell membrane-derived fragments in platelet-free plasma, by ultracentrifugation, and to show that these fragments were capable of generating thrombin. This work showed a linear relationship between the amounts of platelet microparticles (PMP), referred to as "platelet dust", and the platelet count present in the original blood sample. It was also observed that in polycythaemic patients with high platelet counts, there were higher levels of PMP, but low levels in thrombocytopenic patients (Wolf 1967). Even though the first reports on MVs were based on PMP and suggested roles in blood clotting, their ubiquity, as proved in more recent findings, suggests a more general role in various aspects of cell regulation. Despite recent advances, defining MVs remains challenging due to their diversity, and is still open to debate, with different definitions sometimes being adopted by different groups (Shah et al. 2008). Although as we highlighted recently (Hind et al. 2010) MV isolation protocols and analysis parameters are still not standardised, we and others have attempted to improve their accuracy and reliability (Grant et al. 2011).

Microvesicle Composition

The established characteristics for MVs include their size, ~0.1–1 μm in diameter (Deregibus et al. 2007), and for all MVs, regardless of cellular origin, the negatively charged phospholipids such as PS and phosphatidylcholine translocated on to the outer leaflet of the plasma membrane (Scanu et al. 2008) as seen during early apoptosis (Bratton et al. 1997). However, MVs may contain different ratios, or be enriched for phospho- and bioactive-lipids, depending on the type of stimulus or cellular origin (Nolan et al. 2008; Scanu et al. 2008). MVs contain RNAs (mRNA, microRNA and ncRNA) (Deregibus et al. 2007), cytoplasmic components such as enzymes and cytoskeletal proteins (Fox et al. 1990). The cytokines and receptors they carry are characteristic of their cellular origins (Scanu et al. 2008); however, MV composition may undergo substantial remodelling to enable specialised or adaptive functioning (Muralidharan-Chari et al. 2010) or enrichment of particular proteins.

Proteomics using mass spectrometry has become an instrumental tool in the search for new biomarkers. The subproteome of plasma MVs, although recently estimated as 0.01% of the total plasma proteome (Smalley and Ley 2008), is likely to be enriched for certain proteins, e.g. those undergoing unconventional protein export, such as the proinflammatory interleukin (IL)-1β (MacKenzie et al.

2001). Proteomic studies of urinary MVs were recently used to identify biomarkers of nephrotic syndrome (Rood et al. 2010) and urinary exosomes have also been used to identify diagnostic markers for prostate cancer (Nilsson et al. 2009).

We estimated the normal reference range recently (Grant et al. 2011) to be 0.51×10^5 to 2.82×10^5 MVs/ml in blood plasma (Grant et al. 2011). This would equate to approximately one MV being released from 32 cells, assuming $\sim 5 \times 10^6$ cells/ml in blood. This compares with approximately one MV being released from eight cells of a continuous carcinoma cell line, where 1×10^6 cells were stimulated. Although there were insufficient samples, it appeared that a fasting reference range was higher (2.8×10^5 to 5.8×10^5 MVs/ml). Other estimates point to a range of 5–50 μg/ml in the peripheral blood of healthy subjects, which for MVs in the 0.5–0.9 μm size range, gives an estimated range of 2×10^5 to 2×10^6 MVs/ml (Ansa-Addo et al. 2010).

Mechanism of MV Release (Vesiculation)

The exact mechanism which results in membrane vesiculation has not yet been fully elucidated. However, many reports have attributed this to multiple mechanisms, which include both physiological and exogenous agents (Fig. 2). It is important to realise that MVs are not artefacts as sometimes thought or the result of a random process, such as the disintegration of the plasma membrane of dying necrotic cells. Instead, vesiculation is an extremely well-regulated process associated with different cells upon stimulation.

Exogenous stimuli such as epinephrine, adenosine diphosphate, collagen, and calcium ionophore (A23187) have all been reported to stimulate the release of MVs (Horstman and Ahn 1999). In addition to these, sublytic concentrations of the complement membrane attack complex (C5b-9) and mechanical factors such as sheer stress are essential, leading to the release of MVs from various cells. In 2002, it was reported that stimulation of human umbilical vein endothelial cells (HUVECs) with various agents including phorbol myristate acetate, thrombin or tissue plasminogen activator did not result in significant release of PS^+MVs despite changes in cell morphology after activation (Simak et al. 2002). However, an earlier study revealed that HUVECs release PS^+MVs only after stimulation with the lytic complement complex C5b-9, a physiological agonist (Hamilton et al. 1990). Others later reported that these cells release PS^+MVs after stimulation with high concentrations of calcium ionophore, A23187 (10 μM; Qu et al. 2000).

Biochemical Basis of MV Formation

Cell membrane phospholipids are highly specific in terms of their composition and distribution in various eukaryotic cells, under normal physiological conditions most having an asymmetric distribution of membrane phospholipids. The choline phospholipids, phosphatidylcholine and sphingomyelin are mainly localised on the outer leaflet of the plasma membrane, whereas the aminophospholipids, PS and phosphatidylethanolamine are located in the inner layer. The perpetuation of this membrane asymmetry is very important and so it is regulated through a convoluted balance of transmembrane enzymes. The classic theory of plasma membrane blebbing and release of MVs is based on a loss of membrane asymmetry through a transverse migration of anionic phospholipids such as PS from the inner leaflet to the outer leaflet of the plasma membrane (Piccin et al. 2007). This can be stimulated by various cellular events such as apoptosis, necrosis and cell activation. In blood platelets, PS exposure may in turn result in a prothrombotic state (Bevers et al. 1982; Zwaal and Bevers 1983); however, PS exposure also promotes clearance of apoptotic cells (ACs) and cell fragments by macrophages (Borisenko et al. 2003).

An influx of Ca^{2+} from extracellular sources and Ca^{2+} released by the endoplasmic reticulum (ER) causes activation of calpain, a cysteine proteinase of the papainase family. Calpain has several functions during the production of MVs including cleavage of cytoskeletal actin filaments, leading to reorganisation of the cytoskeleton, which facilitates the shedding of MVs. Other functions include activating apoptosis through procaspase 3 and BclxL. To confirm the involvement of calpain in MV release, calpeptin, a known inhibitor of calpain, was shown to inhibit the release of MVs from platelets (Basse et al. 1994). In thrombotic thrombocytopenic purpura, the presence of circulating calpain activity in the plasma is strongly associated with the release of platelet MVs (Kelton et al. 1992).

MVs and Lipid Rafts

It was first thought that translocation of PS from the inner leaflet to the outer leaflet of the plasma membrane represented the only mechanism leading to the release of MVs. However, it is now known that a significant number of MVs released from blood cells and especially endothelial cells does not have accessible PS exposure on the outer leaflet (Shet et al. 2003).

Lipid rafts are specialised membrane domains enriched in certain lipid cholesterols and proteins. These exist as three types, namely caveolae, specialised lipid rafts that

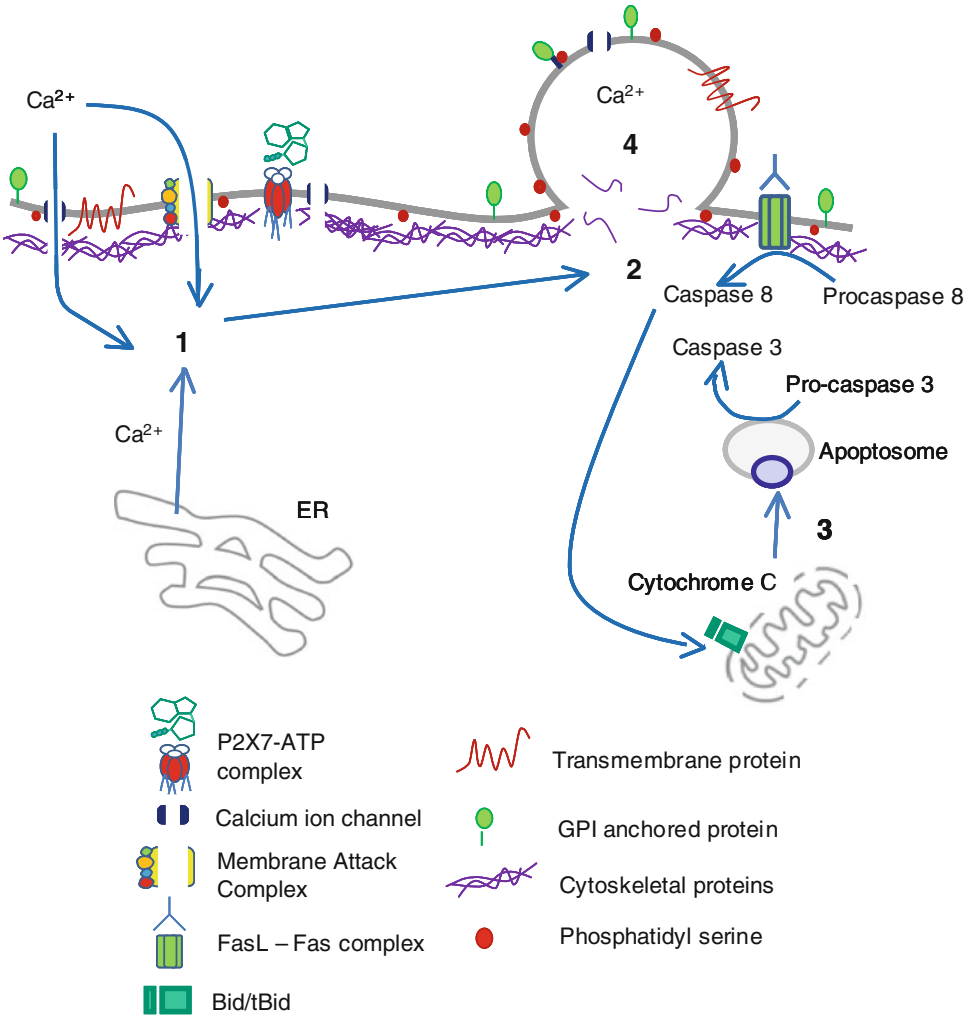

Fig. 2 Cellular pathways to MV release. Ca^{2+} influx via calcium channels, MACs or P2X7-gated calcium channels leads to a microenvironment with increased $[Ca^{2+}]_i$, resulting in the deactivation of cytoskeletal-associated enzymes, membrane instabilities and cytoskeletal protein degradation. Intracellular Ca^{2+} may also originate from organelles, in particular the endoplasmic reticulum (ER). **1** Flippase, floppase and aminophospholipid translocase are unable to maintain membrane asymmetry leading to increased external expression of phosphatidylserine. **2** The simultaneous activation of calpain by free Ca_i^{2+} causes the degradation of the actin cytoskeleton, leading to the formation of the membrane bleb. **3** Mitochondrial stress can lead to MV formation via a different pathway to Ca^{2+} activation. Pro-apoptotic Bcl-2, Bax, Bak insert into the mitochondrial outer membrane causing an increase in membrane permeability leading to reactive oxygen species, cytochrome C and apoptosis inducing factor to leak into the cytoplasm, stimulating the caspase cascade causing cytoskeletal degradation, and the formation of the apoptosome that leads to apoptosis. If the "spill" is relatively small and the damage minimal, the cell can use the apoptosome to form a MV and export the hazardous agents (pseudoapoptosis). Stimulation of FasL leads to the activation of caspase 8 and the subsequent activation of caspase 3, and the translocation of tBid to the mitochondrial membrane, leaking cytochrome C, leading to formation of the apoptosome. The membrane bleb expresses receptors and proteins typical of the parent cell, enriched externally for phosphotidyl serine. **4** Internally, the bleb is enriched for actin and contains a sample of the "local" cytoplasm components such as RNA, protein, peptide hormones and esterases. The bleb also contains undesirable components such as apoptotic agents, notably $Ca^{2+}{}_i$ which is enriched in MVs as a part of the cell's calcium homeostatic mechanisms. The bleb finally pinches off, forming the MV

carry out signalling functions, (glyco) sphingolipid-enriched membranes (GEM), and polyphosphoinositol rich rafts (PIP_2). They can also be subdivided into "inside" rafts (PIP_2 rich and caveolae) and "outside" rafts (GEM) (Jacobson and Dietrich 1999). It was hypothesised recently that lipid rafts may be shed in the form of MVs from the plasma membrane (Simak and Gelderman 2006). The notion that MVs are released from lipid raft domains was further enhanced in a report, which showed that clustering of platelet endothelial cell adhesion molecule-1 (CD31) preceded the shedding of MVs enriched in this molecule on the cell surface (Jy et al. 2002).

Role of MVs as Mediators of Intercellular Communication

The conventional mediators of intercellular communication include cytokines, chemokines, secreted growth factors and various bioactive lipids, nucleotides and nitric oxide ions (Majka et al. 2001; Vandendries et al. 2004). However, it has been speculated for some time as described at various points throughout this review that the MVs (and exosomes) released by cells are able to initiate intercellular communication by the transfer of receptors and their signalling capacities but more recently that they may also be involved in thrombosis (Matzdorff et al. 1998), homeostasis (Nagai et al. 2005), tumour metastasis (Baj-Krzyworzeka et al. 2007), stem cell engraftment (Majka et al. 2007) and angiogenesis (Mostefai et al. 2008).

MV-Mediated Signal Transduction

Recent findings suggest that various biological signals released from parental cells on MVs can be transferred between different cell types (Baj-Krzyworzeka et al. 2002; MacKenzie et al. 2001).

Morphogen-bearing MVs or "argosomes" play essential roles during development, homeostasis and morphogenesis of multicellular organisms. For example, hedgehog morphogens have been shown to be present on MVs derived from activated T cells. Addition of these Hh^+ MVs to human K562 pluripotent erythroleukaemic cells induced their differentiation towards the megakaryocytic lineage, as confirmed by the expression of $\alpha_{IIb}\beta_3$ integrin and CD42b, as well as inducing changes in the cell cycle (Martinez et al. 2006).

In addition, MVs derived from platelets are well documented to activate certain signalling pathways in human cells, including protein kinase C in U937 cells in addition to phosphatidylinositol-3-kinase (PI-3K), mitogen-activated protein kinase (MAPK) p42/44, p38 and c-jun NH_2-terminal kinase 1 (Barry et al. 1999). Others have further showed that MVs from platelets also induced phosphorylation of MAPK p42/44 and PI-3K-AKT signalling pathways, in both normal and malignant human haemopoietic cells (Baj-Krzyworzeka et al. 2002). The activation of MAPK p42/44 and AKT provides further molecular evidence that MVs interact with target cells and activate several signalling cascades that increase cell proliferation and survival.

MV-Mediated Receptor Transfer

The finding that MVs can transfer protein receptors between cells was first studied in platelets. This work showed the transfer, via MVs, of platelet-expressed adhesion molecules to haemopoietic cells, resulting in an increase in their ability to adhere to fibrinogen or endothelium (Janowska-Wieczorek et al. 2001). These reports were supported by an earlier observation that platelet-derived MVs could transfer the CD41 antigen ($\alpha_{IIb}\beta_3$ integrin) to endothelial cells (Barry et al. 1998) as well as to the surface of tumour cells. This transfer increased the adhesiveness of lung cancer cells to the endothelium and also increased their metastasis in vivo after injection into syngeneic mice (Janowska-Wieczorek et al. 2005). Recently, it was also shown that monocyte-derived MVs are not only enriched in P-selectin glycoprotein ligand-1, but also express high levels of tissue factor (Del Conde et al. 2005) which fuses with activated platelets and initiates coagulation after circulating through the peripheral blood. In other studies, it was observed during co-culture of $CD81^+$ Jurkat T cells with $CD81^-$ U937 promonocytic cells that U937 cells became positive for CD81, a tetraspan co-receptor involved in the activation of B and T cells. It was proposed that CD81 was carried on the surface of MVs released by Jurkat cells, and that the receptor had thus been transferred to U937 cells. As a result, the recipient cells were now able to produce an immune response during infection showing retention of receptor function (Fritzsching et al. 2002). MVs isolated from culture supernatants of epithelial ovarian cancer cells were found to express surface Fas ligand (FasL). This ligand normally stimulates apoptosis in T cells, which express the Fas receptor and the secretion of MV-bound FasL which also induces T cell death, thus allowing the tumour cells to escape immunosurveillance (Abrahams et al. 2004).

Another report showed a mechanism by which HIV succeeds in sensitising certain types of cells to infection. The efficient infectivity of haemopoietic cells by HIV-1 is mainly dependent upon host expression of CD4 together with specific virus chemokine co-receptors, which mediate the cell surface binding of the virus, and subsequently facilitates the transmission and propagation of HIV-1 (Berger et al. 1999; Fauci 1996). The principal co-receptor for lymphotropic HIV-1 strains or X4 viruses is the CXCR4 chemokine receptor and the macrophage-tropic HIV-1 strains or R5 viruses use the CCR5 chemokine receptor as co-receptor for entry into cells. The authors observed that individuals who are homozygous for a 32-base pair deletion in the CCR5 gene (Δ32/Δ32), which causes disruption in the expression of the CCR5 receptor, were in fact resistant to HIV infection (Samson et al. 1996). HIV-1 primarily targets haemopoietic cells co-expressing CD4 and chemokine co-receptors. Moreover, there is mounting evidence that other types of cells including endothelial cells (Edinger et al. 1997), astrocytes (Khiati et al. 2010), cardiomyocytes (Eugenin et al. 2008) and

possibly renal cells (Segerer et al. 1999) can be infected with HIV-1 strains and these could be important sources of the virus during chronic infections.

Until recently, there was still debate about how these cells, which do not typically express HIV-related co-receptors on their surface, become susceptible to infection by the virus. This can now be explained through the action of MVs released from cells, which are positive for these co-receptors and their transference to receptor-negative cells. A recent example was that of MVs derived from peripheral blood mononuclear cells transferring specific HIV-1 co-receptors onto endothelial cells during transendothelial migration, rendering these cells susceptible to HIV infection (Mack et al. 2000). A similar mechanism showed that platelet-derived MVs transfer CXCR4 receptor to cells lacking CXCR4 thereby sensitising them to HIV infection (Majka et al. 2000).

In 2005, the pathological roles of released MVs were reported in a study on cerebral malaria (CM) (Combes et al. 2005). This work highlighted the importance of the ATP-binding cassette transporter A1 (ABCA-1), a receptor, which regulates distribution of PS on the outer leaflet of the plasma membrane during MV release. In an experiment using *ABCA-1*$^{-/-}$ mice, the reduced levels of plasma MVs resulted in complete resistance to the development of CM by these mice. By contrast, *ABCA-1*$^{+/+}$ mice released higher numbers of MVs and developed CM when infected by the parasite *Plasmodium berghei* ANKA. Furthermore, MVs isolated from infected mice transferred the receptor to *ABCA-1*$^{-/-}$ mice causing infection by the parasite and development of CM (Combes et al. 2005).

Transfer of HIV, Prions and Mitochondria

In addition to the HIV infection strategy involving transfer of co-receptors, another mechanism involving MVs has been proposed, termed the "Trojan horse mechanism", which elicits the direct transfer of HIV into cells (Gould et al. 2003). A similar mechanism has been hypothesised for prion particle infection of cells as these infectious particles have been observed in platelet-derived MVs. However, further studies are needed to fully understand how far MVs as well as exosomes are involved in the infection process (Bess et al. 1997).

A co-culture of human skin fibroblasts with A549 ρ° cells, a cell line with the mitochondrial DNA mutated and depleted so that the cells became incapable of aerobic respiration and growth, showed that the A549 ρ° cells acquired functional mitochondria from fibroblasts. These cells were able to propagate exponentially in a manner similar to the parental cell line with functional mitochondria (Spees et al. 2006). It is possible that this transfer of functional mitochondria between cells possibly involves MVs and/or exosomes.

Delivering of Proteins, mRNA and microRNA into Target Cells

MVs in general encapsulate some cytoplasm, and thus cytosolic proteins, mRNA and microRNA (miRNA) during their release (Baj-Krzyworzeka et al. 2006; Ratajczak et al. 2006a). Recent studies suggest that co-culturing different cells results in the transfer of membrane and cytoplasmic components between the cultures (Niu et al. 2009). During development, cells undergo functional and molecular changes, which often result in reprogramming of adult cells into pluripotent cells, a process known as epigenetic or nuclear reprogramming (Hochedlinger and Plath 2009). Although the mechanisms responsible are yet to be elucidated, various groups have reported the use of cell extracts to epigenetically reprogramme target cells. Extracts not only from embryonic stem cells (ESC) or pluripotent cancer cells, but also from differentiated somatic cells such as cardiomyocytes have been found to reprogramme terminally differentiated cells (Gaustad et al. 2004; Taranger et al. 2005). Likewise, permeabilised fibroblasts began to express various genes typical of lymphocytes, after being exposed to extracts from lymphocytic cells (Do and Schöler 2004). Despite some reports of MVs and exosomes delivering miRNAs, studies describing a resultant phenotypic change in recipient cells, as described below in the section on MVs in invasive growth and metastasis, in this case promoting angiogenesis (Grange et al. 2011), are rare.

Using a model of murine ESC-derived MVs, it was recently shown that MVs may in fact contribute to epigenetic reprogramming of target cells. Addition of ESC-derived MVs significantly improved the survival and expansion of murine haemopoietic stem and progenitor cells and upregulated the expression of markers for early pluripotent and early haemopoietic stem cells. ESC-derived MVs also induced phosphorylation of MAPK p42/44 and serine–threonine kinase AKT. Furthermore, they observed that ESC-derived MVs were highly enriched in mRNA for various pluripotent transcription factors compared to parent cells, and that this mRNA could be delivered to target cells and translated into corresponding proteins (Ratajczak et al. 2006a).

MV Release as a Possible Secretory Pathway

In eukaryotic cells, a number of specialised organelles assemble sequentially to form a network known as the conventional secretory pathway (Lee et al. 2004). This is a

highly regulated and specialised transport route directly involved in protein biogenesis, modification, sorting, quality control and secretion. The secretory route mediates transport of proteins, lipids and other molecules to intracellular organelles or directly to the plasma membrane where further export to the extracellular space occurs. However, an alternative route has been reported for some proteins, which are exported independent of this secretory pathway, known as the unconventional secretory pathway (Cleves 1997; Nickel 2003, 2005).

MVs Mediate an Unconventional Form of Protein Export

In eukaryotic cells, protein export is mainly via the ER/Golgi-dependent transport or classical secretory pathway. Soluble secreted proteins typified by having an N-terminal, hydrophobic, signal sequence, typically enter this pathway and are translocated across the ER to Golgi and eventually released into the extracellular space. Despite lacking the classical N-terminal, hydrophobic, signal sequence, a number of secretory proteins can still be exported by cells. These proteins do not associate with the classical secretory pathway (Nickel 2003). For example, IL-1β and galectin-1 secretion was observed, despite the absence of a functional ER/Golgi membrane translocation system (Hughes 1999; Rubartelli et al. 1990). These observations have resulted in the term "unconventional protein export", to distinguish secretory processes that are independent of the classical ER/Golgi transport pathway. Such proteins, besides lacking a classical N-terminal, hydrophobic, signal sequence, do not associate with the ER/Golgi membrane translocation machinery and they bear no ER/Golgi-dependent post-translational modifications such as N-linked glycosylation, despite carrying numerous consensus sites. Also, treatment with ER/Golgi transport inhibitors, such as Brefeldin A or monensin, does not affect their export into the extracellular space (Florkiewicz et al. 1995).

Later, others provided evidence that the release of FGF-2 is controlled by NF-κB-mediated signalling and release of another unconventionally exported protein, Engrailed, is regulated by post-translational modifications, such as phosphorylation (Maizel et al. 2002). Additionally, unconventional export of galectins is regulated during cell differentiation (Lutomski et al. 1997). Together, these data indicate that unconventional secretion is not based on unspecific protein release caused by injury, or cell death as previously proposed (McNeil et al. 1989). Instead, it is a highly regulated process involving protein-based molecular machineries.

In general, the majority of unconventional secretory proteins are normally localised to the cytoplasm (Nickel 2003; Prudovsky et al. 2002). However, although they share the properties mentioned above, it is unlikely that their release into the extracellular space is via a common export pathway. Hitherto, four potential export mechanisms have been described in the literature to confer translocation of proteins lacking a classical N-terminal, hydrophobic, signal sequence into the extracellular environment (Hughes 1999), although only two are relevant to this review on MVs and exosomes. The first mechanism involves fusion of multivesicular bodies (MVBs) with the plasma membrane. MVBs carry exosomes, which are packaged with cargo molecules that are released into the extracellular space, as MVBs fuse with the plasma membrane (Stoorvogel et al. 2002). Galectin-3 is an example of an unconventionally secreted protein exported via this route (Thery et al. 2001). The second mechanism is characterised by shedding of MVs into the extracellular environment (Freyssinet 2003; Hugel et al. 2005) as exemplified by the galectin family and as we described for the export of transforming growth factor β1 (TGF-β1) from THP-1 monocytes (Ansa-Addo et al. 2010)

MVs Release Death Signals and Complement Lysis Proteins from Cells

As part of their defensive mechanisms, cells under various stresses release MVs which are often enriched for death signals such as caspases, and Fas which can in turn induce apoptosis in neighbouring cells (Sarkar et al. 2009). Cells can also release MVs to expel deposited membrane attack complex (MAC) and thus escape complement-mediated lysis (Morgan and Campbell 1985). In preliminary submitted work, MV release from *Trypanosoma cruzi* during culture and when stimulated with normal human serum (NHS) has also been examined. If they are shown to have similar mechanisms of interaction with the immune system to their host counterparts, then one could speculate similar roles in terms of escape from complement attack, or acting as decoys to aid their escape from macrophage phagocytosis, so facilitating host cell entry.

Role of MVs in Disease

Besides the link between MVs and a susceptibility to CM mentioned earlier, clinical studies have revealed elevations in the levels of blood MVs in diseases such as atherosclerosis and coronary disorders. Blood from patients with unstable angina or myocardial infarction for example has significant increases in MV levels compared to those having stable angina or non-coronary defects (Mallat et al. 2000). Platelet-derived MVs are elevated in patients with

type 2 diabetes mellitus, but monocyte-derived MVs were even higher in patients with diabetic nephropathy, indicating possible vascular complications in diabetes (Omoto et al. 2002).

The progress already achieved in describing how MV release influences diseases is encouraging. Amongst the conditions in which they have been shown to play a role are cardiovascular diseases (VanWijk et al. 2003), thrombus formation (Furie and Furie 2004) and thrombosis, inflammation and rheumatoid disease (Distler et al. 2005) as well as in sickle cell anaemia, multiple sclerosis and even in pregnancy (Simak et al. 2006). Later studies have suggested that cancer metastasis is enhanced by contact with MVs (Janowska-Wieczorek et al. 2005). Indeed, MVs have recently been shown to enhance angiogenesis (Mostefai et al. 2008), invasion and metastasis of various cancers (Lima et al. 2009; van Doormaal et al. 2009).

MVs and Cancer

Over the last decade, MVs have been shown to play a role directly or indirectly in the pathogenesis of various diseases, cancer being one of them (Amabile et al. 2008; Hsu and Camacho 1999). The presence of MVs in cancer patients had already been noticed since the late 1970s (Friend et al. 1978), and more recently elevated levels of MVs have been found in the blood from cancer patients (Ginestra et al. 1998; Kim et al. 2003; Zwicker et al. 2009).

Various cells have been shown to release MVs as a protective mechanism against intracellular stress as posed by apoptotic enzymes. Caspase 3 is one of the primary enzymes involved in apoptosis in normal cell physiology, and recently various researchers have reported the presence of this enzyme in the conditioned medium of viable cell cultures (Sapet et al. 2006; Böing et al. 2008) much of which is carried by MVs. It is thought that the cells release caspase 3 via MVs in order to prevent intracellular accumulation and thus escape apoptosis. This hypothesis was strengthened by the finding that inhibiting MV release allows caspase 3 to accumulate in cells which then undergo apoptosis (Abid Hussein et al. 2007). As cancer cells retain the majority of cellular functions of their non-cancerous counterparts, elevated MV release may be one way by which cancer cells escape cell death.

Cancer cells have also been shown to be resistant to a variety of chemotherapeutic drugs. Cancer cells insensitive to chemotherapy express more genes related to membrane shedding as opposed to chemo-sensitive cells. Interestingly, MVs from chemo-insensitive cells contain high levels of chemotherapeutic drugs such as Doxorubicin (Shedden et al. 2003). A similar study was performed on another resistant chemotherapeutic drug cisplatin. This demonstrated that cells insensitive to cisplatin released MVs containing about 2.5-fold more cisplatin than MVs released from sensitive cells (Safaei et al. 2005), implying that cancer cells probably eject the chemotherapeutic drug through the increased release of MVs in order to escape drug-induced apoptosis and possibly providing a mechanism for multidrug resistance.

Cancer cells are also able to escape immuno-surveillance. Although the tumour environment is well known to be resistant to complement attack, micro-tumours are more susceptible to complement. The concept that cells exposed to complement C5b-9 complex (MAC) survived by releasing MVs rich in MAC was proposed in 1988 (Sims et al. 1988). Various researchers have shown since then that cancer cells use the same mechanism to evade the complement system (Whitlow and Klein 1997). The mechanism was further enhanced by the finding that cancer cells shed MVs rich in the complement inhibitor protein CD46, which inactivates complement C3b and C4b proteins (Hakulinen et al. 2004). Cancer cells are thus able not only to prevent complement from attacking, but also to inactivate it.

Suppression of the immune system is achieved by cancer cells releasing MVs rich in immuno-modulatory molecules such as FasL (thereby diminishing the function of the adaptive immune system), and latent membrane protein-I which inhibits leukocyte proliferation (Andreola et al. 2002; Huber et al. 2005). MVs also enable the exchange of surface lipids and proteins such as integrins which in turn mask the cells as non-cancerous thereby escaping immuno-surveillance (Janowska-Wieczorek et al. 2005, 2006; Valenti et al. 2006).

The Role of MVs in Invasive Growth and Metastasis

After successfully evading the immune system, not only do cancers proliferate and grow into large tumours, they also spread to other parts of the body through metastasis. For a tumour to migrate, it must degrade the extracellular matrix (ECM). In a physiological setting, this is achieved by proteases such as matrix metalloproteinases 2 (MMP-2) and MMP-9 as well as urokinase plasminogen activator (uPA) which would degrade the basement membrane collagen, as well as various components of the ECM such as fibrin; it is now thought that cancer cells break down the ECM through the release of MVs rich in MMP-2 and MMP-9 as well as uPA (Graves et al. 2004). These workers reported elevated levels of MVs rich in MMPs and uPA from patients with late stage as opposed to early stage ovarian carcinoma and when these MV-bound enzymes were inhibited this virtually eliminated the ability of the MVs to support tumour invasiveness (Graves et al. 2004).

Together with ECM degradation, MVs have also been shown to aid cancer cells in angiogenesis. It is a well-established fact that not only do cancer patients have elevated levels of MVs in their blood, but also these MVs are rich in tissue factor, a procoagulant factor contributing to fibrin formation. The fibrin eventually protects the tumour against the immune system and forms a matrix to support angiogenesis (Rauch and Antoniak 2007). This procoagulant effect of MVs has also been shown to indirectly activate the release of growth factors such as vascular endothelial growth factor (VEGF). One way by which cancer cell-derived MVs could be likely to activate growth factors is through MVs carrying mRNA encoding growth factors such as VEGF and hepatocyte growth factor. Upon fusion of the MVs with monocytes, transfer of nucleic acid material results in induction of these growth factors (Baj-Krzyworzeka et al. 2006, 2007). Whether or not MVs promote cancer cell metastasis has not yet been studied extensively. However, one can postulate that the MVs probably aid in cancer metastasis indirectly, through the immunosuppressive and angiogenic effects of the cancer cell-derived MVs, as seen earlier.

MVs are also able to transmit proteins such as tissue transglutaminase and fibronectin from breast and brain cancer cells to normal fibroblasts and epithelial cells, resulting in their transformation (Antonyak et al. 2011). MVs released from cancer stem cells ($CD105^{+}$) in human renal cell carcinoma stimulate angiogenic properties in endothelial cells in vitro (Grange et al. 2011). Upon transfer to a mouse model of renal carcinoma in immunocompromised mice, these MVs carrying proangiogenic miRNAs and mRNAs stimulate angiogenesis resulting in establishment of a premetastatic niche in lung.

Hypoxia-Mediated Microvesiculation in Tumours and Stroke

Non-apoptosis-inducing doses of hypoxia (1% O_2) induce microvesiculation of human lung cancer cell lines (A549) (Wysoczynski and Ratajczak 2009), which in turn promotes angiogenesis through promoting chemotaxis of fibroblasts and endothelial cells and stimulates a release of pro-angiopoietic cytokines from stromal cells. Mouse models of metastasis using A549 cells further show that such tumour MVs derived from lung cancer can stimulate stroma fibroblasts thereby enhancing metastasis and tumour progression. Similar effects, this time of tumour-derived exosomes, from hypoxic epidermoid carcinoma cells, A431, showed similar enhancement of angiogenesis in a chorioallantoic membrane assay using a chick embryo model and in promoting metastatic potential (Park et al. 2010).

Other studies have focused on cerebral hypoxia induced by acute ischemic stroke. The first, analysing 90 cases of stroke showed significantly increased levels of platelet-derived MVs which persisted for up to 6 months after stroke and which was speculated might represent a marker of risk of stroke (Cherian et al. 2003). A more recent study has shown certain endothelial MVs to be at elevated levels in acute ischemic stroke which correlate with stroke pathophysiology and outcome (Simak et al. 2006). Interestingly, MVs, especially exosomes, have been considered as potential delivery vehicles of drugs and in experiments targeting mouse brain, it has been possible to deliver siRNA by systemic injection specifically targeting brain neurons, microglia and oligodendrocytes (Alvarez-Erviti et al. 2011).

MVs and Infectious Disease

Hitherto, exosomes have been suggested to "hijack" infectious particles such as HIV or prions from the cytoplasm (Pelchen-Matthews et al. 2004; Robertson et al. 2006) and even whole intact organelles such as mitochondria (Spees et al. 2006). As mentioned earlier, in addition to stimulating mammalian cells to secrete vesicles, parasites could themselves release MVs although the reason(s) for this can only be speculated upon at present. In our recent work (Ansa-Addo et al. 2012; Cestari et al. 2012), we provide evidence that MVs isolated from THP-1 cells fuse with *T. cruzi* and increase their invasion of Vero and HeLa cells by evading complement lysis. Furthermore, MVs isolated from the intestinal parasite, *Giardia intestinalis* (causative agent of giardiasis and persistent non-bacterial diarrhoea), enhanced the parasite's attachment to endothelial cells (unpublished observations). The attachment of *G. intestinalis* to the microvilli of endothelial cells is necessary for the parasite to effectively establish infection (Hansen and Fletcher 2008). This datum is the first to report possible contributions of MVs during parasite infections. In other preliminary observations from our lab in which *Salmonella typhimurium* was used to infect Vero cells, it was found that in the presence of MVs (Fig. 3), there was a significantly increased level of infection.

MVs and Cellular Differentiation

Our recent study (Ansa-Addo et al. 2010) demonstrated for the first time that MVs (isolated from THP-1 monocytic cells) carry TGF-β1 on their membrane surface. These TGF-β1-bearing MVs could modulate the growth rate of peripheral blood monocytes and their leukemic counterparts in vitro without stimulating apoptosis. In addition, the

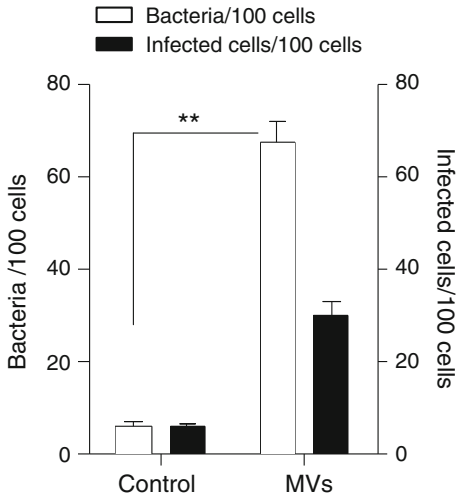

Fig. 3 Infection of Vero cells with *Salmonella typhimurium* is markedly enhanced in the presence of MVs derived from THP-1 monocytes. Semiconfluent Vero cells in serum-free medium were infected in vitro with *S. typhimurium* strain SL1344. Vero cells were infected at a 5:1 ratio (bacteria to cell) for 3 h and incubated at 37°C in a humidified atmosphere of 5% CO_2. Cells were then washed three times with PBS, fixed for 10 min at RT with 4% paraformaldehyde and washed a further three times with PBS. Coverslips were mounted on microscope slides with DAPI-Vectashield medium. The number of bacteria was determined by counting at least 300 cells. To check for multiple infections, the number of infected cells was also counted. Data were normalized to 100

cells completely attached to the culture plate, and expressed typical markers of macrophages including CD14 and CD11b, but not the dendritic cell-specific marker, DC-SIGN (Tailleux et al. 2005). It was also found that MVs isolated from Jurkat and THP-1 cells carrying high TGF-β1 (>200 pg/10^5 MVs) caused significantly greater reductions in the growth rate of THP-1 cells, as compared to MVs from MCF-7 breast cancer cells expressing lower levels of TGF-β1 ($\sim$30 pg/10^5 MVs).

Previously, it had been shown that recombinant TGF-β1 could promote differentiation of human promonocytic leukemic cells into macrophages (Bombara and Ignotz 1992). The work of Ansa-Addo et al. (2010) was in agreement but went on to show that upon attachment of MVs to THP-1 cells, which may as in the case of contact (and fusion) of monocyte-derived MVs with platelets be annexin V-mediated, the source of macrophage-inducing TGF-β1 was that carried on MVs. Although contact with or phagocytosis of ACs and MVs by macrophages has been shown to release TGF-β1, at levels of 80 pg/ml (Fadok et al. 1998) and 350 pg/ml (Gasser and Schifferli 2004), respectively, importantly, such release and autocrine-like action of TGF-β1 on the THP-1 cells, resulting in the observed reduction in proliferation (and consequent differentiation), is unlikely to have occurred in this study, as low TGF-β1-carrying MCF-7-derived MVs were unable to elicit significant reductions in proliferation.

Several inhibitors of TGF-β1 signalling have been reported as potential therapeutics in cancer immunotherapy. Amongst these is the TGF-β receptor antagonist, SB-431542, shown to inhibit the ligand-dependent growth of HT-29 colon cancer cells (Halder et al. 2005). It was also demonstrated to be a selective inhibitor of endogenous activin and TGF-β1 signalling (Yingling et al. 2004). The involvement of TGF-β1 was demonstrated in this study by showing that SB-431542 almost completely reversed the growth inhibition due to MV treatment of cells.

MVs in Autoimmune Disease

The effective removal of ACs by phagocytes is not just limited to safe removal of injured, aged or infected cells but is crucial to processes ranging from embryogenesis, tissue homeostasis, and cellular immunity to the resolution of inflammation (Albert et al. 2000). It has also been well established that in certain clinical conditions such as cystic fibrosis as well as chronic autoimmune diseases (Brechot et al. 2008), there is a defect in the clearance of ACs. Various studies have shown that MVs express high concentrations of PS on the outer leaflet of the plasma membrane when they are released via blebbing and shedding following the loss of phospholipid membrane asymmetry on the parent cell (Butikofer et al. 1989). Our recent work shows that MVs, by competing with ACs, may play a role in the reduction of phagocytosis of ACs by macrophages, so contributing to autoimmune disease (Antwi-Baffour et al. 2010).

ACs also express PS on the outer leaflet, this being one of the main signals for their phagocytosis by macrophages (Brüne 2003). Blocking of PS interaction with PS receptor, on phagocytes with a PS receptor antibody, inhibits the phagocytosis of ACs significantly, whilst enrichment of cell membranes with exogenous PS results in the phagocytic clearance of Jurkat cells (Chen and Goeddel et al. 2002). We hypothesised that the dose-dependent reduction in phagocytosis by MVs observed could be due to PS on MVs binding to PS receptors on macrophages thereby directly competing with PS on ACs. This was confirmed by blocking the PS on MVs with annexin V, which abrogated MVs' inhibition of AC phagocytosis. In addition to anti-phospholipid antibody (aPLA) plasma samples having been shown to stimulate higher levels of MV release from Jurkat cells, plasma MV levels in systemic lupus erythematosus (SLE) were also higher than those of non-SLE plasma. Also depletion of IgG or complement C9 from NHS reduced MV release and this was partially restored upon addition of purified aPLA (1 mg/ml) to the NHS.

Our findings do have certain clinical implications. It has been well established that increased apoptosis occurs in a variety of clinical settings such as during chemotherapy and radiation therapy (Cheng et al. 2003; Chiarugi and Moskowitz 2002) as well as in certain autoimmune diseases such as rheumatoid arthritis and SLE and that in the latter there is impaired phagocytosis of ACs (Herrmann et al. 1998). In addition to this, ACs themselves have been shown to produce MVs which in turn can induce further MV production (Cohen et al. 1992). In these cases, the already high level of ACs would rise still further as more AC-derived MVs would reduce the phagocytosis of ACs leading to their secondary necrosis, thereby intensifying inflammation, cell injury and worsening the disease prognosis.

Conclusion

In recent years, extensive work in various disciplines has shown MVs to be important mediators of intercellular communication with roles in normal cell physiology as well as in infectious disease, cancer and autoimmune disease. It is now time to think of the applications MVs can be used in to aid various areas of biomedicine. Their interaction with cells means they may show potential as novel therapeutic agents. However, how they could be targeted to particular cellular targets yet remains to be elucidated, although in certain cases modulation of MV secretion or sometimes increased expression may also be applicable. Despite the plethora of studies on MVs and exosomes in the last 5 years, most have been conducted in vitro on cell lines. To show their relevance in disease will require studies using animal models of disease, as exemplified by the work using an ABCA1$^{-/-}$ mouse, deficient in plasma MVs, to show their importance in CM. Finally, future studies are likely to lead to novel immunodiagnostic techniques involving MVs in for example diagnosing infectious disease or cancer.

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References

Abid Hussein MN, Böing AN, Sturk A et al (2007) Inhibition of microparticle release triggers endothelial cell apoptosis and detachment. Thromb Haemost 98:1096–1107

Abrahams VM, Straszewski-Chavez SL, Guller S (2004) First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. Mol Hum Reprod 10:55–63

Albert ML, Kim JI, Birge RB (2000) Alphavbeta5 intregrin recruits the crkII-Dock180-rac1 complex for phagocytosis of apoptotic cells. Nat Cell Biol 2:899–905

Alvarez-Erviti L, Seow Y, Yin H et al (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29:341–345

Amabile N, Heiss C, Real WM et al (2008) Circulating endothelial microparticle levels predict hemodynamic severity of pulmonary hypertension. Am J Respir Crit Care Med 177:1268–1275

Andreola G, Rivoltini L, Castelli C et al (2002) Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. J Exp Med 195:1303–1316

Ansa-Addo EA, Lange S, Stratton D et al (2010) Human plasma membrane-derived vesicles halt proliferation and induce differentiation of THP-1 acute monocytic leukaemia cells. J Immunol 185:5236–5246

Ansa-Addo EA, Cestari I, Pathak P et al (2012) *Trypanosoma cruzi* utilisation of host cell microvesicle release aids invasion. J Immunol (submitted)

Antonyak MA, Li B, Boroughs LK et al (2011) Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. Proc Natl Acad Sci USA 108:4852–4857

Antwi-Baffour S, Kholia S, Aryee YK-D et al (2010) Human plasma membrane-derived vesicles inhibit the phagocytosis of apoptotic cells—possible role in SLE. Biochem Biophys Res Commun 398:278–283

Aoki N, Yokoyama R, Asai N et al (2010) Adipocyte-derived microvesicles are associated with multiple angiogenic factors and induce angiogenesis in vivo and in vitro. Endocrinology 151:2567–2576

Baj-Krzyworzeka M, Majka M, Pratico D et al (2002) Platelet-derived microparticles stimulate proliferation, survival, adhesion, and chemotaxis of hematopoietic cells. Exp Hematol 30:450–459

Baj-Krzyworzeka M, Szatanek R, Weglarczyk K et al (2006) Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. Cancer Immunol Immunother 55:808–818

Baj-Krzyworzeka M, Szatanek R, Weglarczyk K et al (2007) Tumour-derived microvesicles modulate biological activity of human monocytes. Immunol Lett 113:76–82

Barry OP, Kazanietz MG, Pratico D et al (1999) Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. J Biol Chem 274:7545–7556

Barry OP, Pratico D, Savani RC et al (1998) Modulation of monocyte-endothelial cell interactions by platelet microparticles. J Clin Invest 102:136–144

Basse F, Gaffet P, Bienvenue A (1994) Correlation between inhibition of cytoskeleton proteolysis and anti-vesiculation effect of calpeptin during A23187-induced activation of human platelets: are vesicles shed by filopod fragmentation? Biochim Biophys Acta 1190:217–224

Berger EA, Murphy PM, Farber JM (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 17:657–700

Bess JW Jr, Gorelic RJ, Bosche WJ et al (1997) Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. Virology 230:134–144

Bevers EM, Comfurius P, van Rijn JL et al (1982) Generation of prothrombin-converting activity and the exposure of phosphatidylserine at the outer surface of platelets. Eur J Biochem 122:429–436

Böing N, Hau CM, Sturk A et al (2008) Platelet microparticles contain active caspase 3. Platelets 19:96–103

Bombara C, Ignotz RA (1992) TGF-beta inhibits proliferation of and promotes differentiation of human promonocytic leukemia cells. J Cell Physiol 153:30–37

Borisenko GG, Matsura T, Liu SX et al (2003) Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic Jurkat cells—existence of a threshold. Arch Biochem Biophys 413:41–52

Bratton DL, Fadok VA, Richter DA et al (1997) Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. J Biol Chem 272:26159–26165

Brechot N, Gomez E, Bignon M et al (2008) Modulation of macrophage activation state protects tissue from necrosis during critical limb ischemia in thrombospondin-1-deficient mice. PLoS One 3:e3950

Brüne B (2003) Nitric oxide: NO apoptosis or turning it ON? Cell Death Differ 10:864–869

Butikofer P, Kuypers FA, Xu CM et al (1989) Enrichment of two glycosylphosphatidylinositol-anchored proteins, acetylcholinesterase and decay accelerating factor, in vesicles released from human red blood cells. Blood 74:1481–1485

Cestari I, Ansa-Addo E, Deolindo P et al (2012) *Trypanosoma cruzi* immune evasion mediated by host cell-derived microvesicles. J Immunol 188:1942–1952

Chargaff E, West R (1946) The biological significance of the thromboplastic protein of blood. J Biol Chem 166:189–197

Chen JF, Goeddel DV (2002) TNF-R1 signaling: a beautiful pathway. Science 296:1634–1635

Cheng EH, Sheiko TV, Fisher JK et al (2003) VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 301:513–517

Cherian P, Hankey GJ, Eikelboom JW et al (2003) Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. Stroke 34:2132–2137

Chiarugi A, Moskowitz MA (2002) Cell biology. PARP-1—a perpetrator of apoptotic cell death? Science 297:200–201; comments 259–263

Cleves AE (1997) Protein transports: the nonclassical ins and outs. Curr Biol 7:R318–R320

Cohen JJ, Duke RC, Fadok VA et al (1992) Apoptosis and programmed cell death in immunity. Annu Rev Immunol 10:267–293

Combes V, Coltel N, Alibert M et al (2005) *ABCA1* gene deletion protects against cerebral malaria: potential pathogenic role of microparticles in neuropathology. Am J Pathol 166:295–302

Del Conde I, Shrimpton CN, Thiagarajan P et al (2005) Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood 106:1604–1611

Deregibus MC, Cantaluppi V, Calogero R et al (2007) Endothelial progenitor cell-derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110:2440–2448

Distler JH, Pisetsky DS, Huber LC et al (2005) Microparticles as regulators of inflammation: novel players of cellular crosstalk in the rheumatic diseases. Arthritis Rheum 52:3337–3348

Do JT, Schöler HR (2004) Nuclei of embryonic stem cells reprogram somatic cells. Stem Cells 22:941–949

Edinger AL, Mankowski JL, Doranz BJ et al (1997) CD4-independent, CCR5-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. Proc Natl Acad Sci USA 94:14742–14747

Eugenin EA, Morgello S, Klotman ME et al (2008) Human immunodeficiency virus (HIV) infects human arterial smooth muscle cells in vivo and in vitro: implications for the pathogenesis of HIV-mediated vascular disease. Am J Pathol 172:1100–1111

Fadok VA, Bratton DL, Konowal A et al (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 101:890–898

Fauci AS (1996) Host factors and the pathogenesis of HIV-induced disease. Nature 384:529–534

Florkiewicz RZ, Majack RA, Buechler RD et al (1995) Quantitative export of FGF-2 occurs through an alternative, energy-dependent, non-ER/Golgi pathway. J Cell Physiol 162:388–399

Fox JE, Austin CD, Boyles JK et al (1990) Role of the membrane skeleton in preventing the shedding of procoagulant-rich microvesicles from the platelet plasma membrane. J Cell Biol 111:483–493

Freyssinet JM (2003) Cellular microparticles: what are they bad or good for? J Thromb Haemost 1:1655–1662

Friend C, Marovitz W, Henie G et al (1978) Observations on cell lines derived from a patient with Hodgkin's disease. Cancer Res 38:2581–2591

Fritzsching B, Schwer B, Kartenbeck J et al (2002) Release and intercellular transfer of cell surface CD81 via microparticles. J Immunol 169:5531–5537

Furie B, Furie BC (2004) Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. Trends Mol Med 10:171–178

Gasser O, Hess C, Miot S et al (2003) Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. Exp Cell Res 285:243–257

Gasser O, Schifferli JA (2004) Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. Blood 104:2543–2548

Gaustad KG, Boquest AC, Anderson BE (2004) Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. Biochem Biophys Res Commun 314:420–427

George JN, Thoi LL, McManus LM et al (1982) Isolation of human platelet membrane microparticles from plasma and serum. Blood 60:834–840

Ginestra A, La Placa MD, Saladino F et al (1998) The amount and proteolytic content of vesicles shed by human cancer cell lines correlates with their in vitro invasiveness. Anticancer Res 18:3433–3437

Gould SJ, Booth AM, Hildreth JE (2003) The Trojan exosome hypothesis. Proc Natl Acad Sci USA 100:10592–10597

Grange C, Tapparo M, Collino F et al (2011) Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res 71:5346–5356

Grant R, Ansa-Addo EA, Stratton D et al (2011) A filtration-based protocol to isolate human plasma membrane-derived vesicles and exosomes from blood plasma. J Immunol Methods 371:143–151

Graves LE, Ariztia EV, Navari JR et al (2004) Proinvasive properties of ovarian cancer ascites-derived membrane vesicles. Cancer Res 64:7045–7049

Hakulinen J, Junnikkala S, Sorsa T et al (2004) Complement inhibitor membrane cofactor protein (MCP; CD46) is constitutively shed from cancer cell membranes in vesicles and converted by a metalloproteinase to a functionally active soluble form. Eur J Immunol 34:2620–2629

Halder SK, Beauchamp RD, Datta PK (2005) A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. Neoplasia 7:509–521

Hamilton KK, Hattori R, Esmon CT et al (1990) Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 265:3809–3814

Hansen WR, Fletcher DA (2008) Tonic shock induces detachment of *Giardia lamblia*. PloS Negl Trop Dis 2:e169

Herrmann M, Voll RE, Zoller OM et al (1998) Impaired phagocytosis of apoptotic cell material by monocyte derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum 41:1241–1250

Hess C, Sadallah S, Hefti A et al (1999) Ectosomes released by human neutrophils are specialized functional units. J Immunol 163:4564–4573

Hind E, Heugh S, Ansa-Addo EA et al (2010) Red cell PMVs, plasma membrane-derived vesicles calling out for standards. Biochem Biophys Res Commun 399:465–469

Hochedlinger K, Plath K (2009) Epigenetic reprogramming and induced pluripotency. Development 136:509–523

Horstman LL, Ahn YS (1999) Platelet microparticles: a wide-angle perspective. Crit Rev Oncol Hematol 30:111–142

Hsu HH, Camacho NP (1999) Isolation of calcifiable vesicles from human atherosclerotic aortas. Atherosclerosis 143:353–362

Huber V, Fais S, Iero M et al (2005) Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. Gastroenterology 128:1796–1804

Hugel B, Martinez MC, Kunzelmann C et al (2005) Membrane microparticles: two sides of the coin. Physiology 20:22–27

Hughes RC (1999) Secretion of the galectin family of mammalian carbohydrate-binding proteins. Biochim Biophys Acta 1473:172–185

Jacobson K, Dietrich C (1999) Looking at lipid rafts? Trends Cell Biol 9:87–91

Janowska-Wieczorek A, Majka M, Kijowski J et al (2001) Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. Blood 98:3143–3149

Janowska-Wieczorek A, Marquez-Curtis LA, Wysoczynski M et al (2006) Enhancing effect of platelet-derived microvesicles on the invasive potential of breast cancer cells. Transfusion 46:1199–1209

Janowska-Wieczorek A, Wysoczynski M, Kijowski J et al (2005) Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. Int J Cancer 113:752–760

Jy W, Jimenez JJ, Mauro LM et al (2002) Agonist-induced capping of adhesion proteins and microparticle shedding in cultures of human renal microvascular endothelial cells. Endothelium 9:179–189

Kelton JG, Warkentin TE, Hayward CP et al (1992) Calpain activity in patients with thrombotic thrombocytopenic purpura is associated with platelet microparticles. Blood 80:2246–2251

Khiati A, Chaloin O, Muller S et al (2010) Induction of monocyte chemoattractant protein-1 (MCP-1/CCL2) gene expression by human immunodeficiency virus-1 Tat in human astrocytes is CDK9 dependent. J Neurovirol 16:150–167

Kim HK, Song KS, Park YS et al (2003) Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. Eur J Cancer 39:184–191

Kim JW, Wieckowski E, Taylor DD et al (2005) Fas ligand-positive membranous vesicles isolated form sera of patients with oral cancer induce apoptosis of activated T lymphocytes. Clin Cancer Res 11:1010–1020

Lee MC, Miller EA, Goldberg J et al (2004) Bi-directional protein transport between the ER and Golgi. Annu Rev Cell Dev Biol 20:87–123

Lima LG, Chammas R, Monteiro RQ et al (2009) Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. Cancer Lett 283:168–175

Lutomski D, Fouillit M, Bourin P et al (1997) Externalization and binding of galectin-1 on cell surface of K562 cells upon erythroid differentiation. Glycobiology 7:1193–1199

Mack M, Kleinschmidt A, Bruhl H et al (2000) Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. Nat Med 6:769–775

MacKenzie A, Wilson HL, Kiss-Toth E et al (2001) Rapid secretion of interleukin-1beta by microvesicle shedding. Immunity 15:825–835

McNeil PL, Muthukrishnan L, Warder E et al (1989) Growth factors are released by mechanically wounded endothelial cells. J Cell Biol 109:811–822

Maizel A, Tassetto M, Filhol O et al (2002) Engrailed homeoprotein secretion is a regulated process. Development 129:3545–3553

Majka M, Janowska-Wieczorek A, Ratajczak J et al (2001) Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. Blood 97:3075–3085

Majka M, Kijowski J, Lesko E et al (2007) Evidence that platelet-derived microvesicles may transfer platelet-specific immunoreactive antigens to the surface of endothelial cells and CD34+ hematopoietic stem/progenitor cells—implication for the pathogenesis of immune thrombocytopenias. Folia Histochem Cytobiol 45:27–32

Majka M, Rozmyslowicz T, Honczarenko M et al (2000) Biological significance of the expression of HIV-related chemokine coreceptors (CCR5 and CXCR4) and their ligands by human hematopoietic cell lines. Leukemia 14:1821–1832

Mallat Z, Benamer H, Hugel B et al (2000) Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 101:841–843

Martinez MC, Larbret F, Zobairi F et al (2006) Transfer of differentiation signal by membrane microvesicles harboring hedgehog morphogens. Blood 108:3012–3020

Matzdorff AC, Berchner D, Kuhnel G et al (1998) Relative and absolute changes of activated platelets, microparticles and platelet aggregates after activation in vitro. Haemostasis 28:277–288

Morgan BP, Campbell AK (1985) The recovery of human polymorphonuclear leucocytes from sublytic complement attack is mediated by changes in intracellular free calcium. Biochem J 231:205–208

Mostefai HA, Andriantsitohaina R, Martinez MC (2008) Plasma membrane microparticles in angiogenesis: role in ischemic diseases and in cancer. Physiol Res 57:311–320

Muralidharan-Chari V, Clancy JW, Sedgwick A et al (2010) Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci 123:1603–1611

Nagai A, Sato T, Akimoto N et al (2005) Isolation and identification of histone H3 protein enriched in microvesicles secreted from cultured sebocytes. Endocrinology 146:2593–2601

Nickel W (2003) The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. Eur J Biochem 270:2109–2119

Nickel W (2005) Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells. Traffic 6:607–614

Nilsson J, Skog J, Nordstrand A et al (2009) Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer 100:1603–1607

Niu X, Gupta K, Yang JT et al (2009) Physical transfer of membrane and cytoplasmic components as a general mechanism of cell–cell communication. J Cell Sci 122:600–610

Nolan S, Dixon R, Norman K et al (2008) Nitric oxide regulates neutrophil migration through microparticle formation. Am J Pathol 172:265–273

Omoto S, Nomura S, Shouzu A et al (2002) Detection of monocyte-derived microparticles in patients with Type II diabetes mellitus. Diabetologia 45:550–555

Pap E, Pallinger E, Pasztoi M et al (2009) Highlights of a new type of intercellular communication: microvesicle-based information transfer. Inflamm Res 58:1–8

Park JE, Tan HS, Datta A et al (2010) Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. Mol Cell Proteomics 9:1085–1099

Pelchen-Matthews A, Raposo G, Marsh M (2004) Endosomes, exosomes and Trojan viruses. Trends Microbiol 12:310–316

Piccin A, Murphy WG, Smith OP (2007) Circulating microparticles: pathophysiology and clinical implications. Blood Rev 21:157–171

Prudovsky I, Bagala C, Tarantini F et al (2002) The intracellular translocation of the components of the fibroblast growth factor 1 release complex precedes their assembly prior to export. J Cell Biol 158:201–208

Putz U, Howitt J, Lackovic J et al (2008) Nedd4 family-interacting protein 1 (Ndfip1) is required for the exosomal secretion of Nedd4 family proteins. J Biol Chem 283:32621–32627

Qu J, Adam J, Bloxham DM et al (2000) Phosphatidylserine-dependent adhesion of T cells to endothelial cells. Biochim Biophys Acta 1501:99–115

Ratajczak J, Miekus K, Kucia M et al (2006a) Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia 20:847–856

Ratajczak J, Wysoczynski M, Hayek F et al (2006b) Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia 20:1487–1495

Rauch U, Antoniak S (2007) Tissue factor-positive microparticles in blood associated with coagulopathy in cancer. Thromb Haemost 97:9–10

Robertson C, Booth SA, Beniac DR et al (2006) Cellular prion protein is released on exosomes from activated platelets. Blood 107:3907–3911

Rood IM, Deegens JK, Merchant ML et al (2010) Comparison of three methods for isolation of urinary microvesicles to identify biomarkers of nephrotic syndrome. Kidney Int 78:810–816

Rubartelli A, Cozzolino F, Talio M et al (1990) A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. EMBO J 9:1503–1510

Sadallah S, Eken C, Schifferli JA (2008) Erythrocyte-derived ectosomes have immunosuppressive properties. J Leukoc Biol 84:1316–1325

Safaei R, Larson BJ, Cheng TC et al (2005) Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Mol Cancer Ther 4:1595–1604

Samson M, Libert F, Doranz BJ et al (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the *CCR-5* chemokine receptor gene. Nature 382:722–725

Sapet C, Simoncini S, Loriod B et al (2006) Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. Blood 108:1868–1876

Sarkar A, Mitra S, Mehta S et al (2009) Monocyte derived microvesicles deliver a cell death message via encapsulated caspase-1. PLoS One 4:e7140

Satta N, Toti F, Feugeas O et al (1994) Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. J Immunol 153:3245–3255

Scanu A, Molnarfi N, Brandt KJ et al (2008) Stimulated T cells generate microparticles, which mimic cellular contact activation of human monocytes: differential regulation of pro- and anti-inflammatory cytokine production by high-density lipoproteins. J Leukoc Biol 83:921–927

Scholz T, Temmler U, Krause S et al (2002) Transfer of tissue factor from platelets to monocytes: role of platelet-derived microvesicles and CD62P. Thromb Haemost 88:1033–1038

Segerer S, Mack M, Regele H et al (1999) Expression of the C-C chemokine receptor 5 in human kidney diseases. Kidney Int 56:52–64

Shah MD, Bergeron AL, Dong JF et al (2008) Flow cytometric measurement of microparticles: pitfalls and protocol modifications. Platelets 19:365–372

Shedden K, Xie XT, Chandaroy P et al (2003) Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. Cancer Res 63:4331–4337

Shet AS, Aras O, Gupta K et al (2003) Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. Blood 102:2678–2683

Simak J, Gelderman MP (2006) Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers. Transfus Med Rev 20:1–26

Simak J, Gelderman MP, Yu H et al (2006) Circulating endothelial microparticles in acute ischemic stroke: a link to severity, lesion volume and outcome. J Thromb Haemost 4:1296–1302

Simak J, Holada K, Vostal JG (2002) Release of annexin V-binding membrane microparticles from cultured human umbilical vein endothelial cells after treatment with camptothecin. BMC Cell Biol 3:11

Sims PJ, Faioni EM, Wiedmer T et al (1988) Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. J Biol Chem 263:18205–18212

Smalley DM, Ley K (2008) Plasma-derived microparticles for biomarker discovery. Clin Lab 54:67–79

Spees JL, Olson SD, Whitney MJ et al (2006) Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci USA 103:1283–1288

Stoorvogel W, Kleijmeer MJ, Geuze HJ et al (2002) The biogenesis and functions of exosomes. Traffic 3:321–330

Tailleux L, Pham-Thi N, Bergeron-Lafaurie A et al (2005) DC-SIGN induction in alveolar macrophages defines privileged target host cells for mycobacteria in patients with tuberculosis. PLoS Med 2:e381

Taranger CK, Noer A, Sorensen AL et al (2005) Induction of dedifferentiation, genomewide transcriptional programming, and epigenetic reprogramming by extracts of carcinoma and embryonic stem cells. Mol Biol Cell 16:5719–5735

Thery C, Boussac M, Veron P et al (2001) Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 166:7309–7318

Tomas A, Yermen B, Min L et al (2006) Regulation of pancreatic β-cell insulin secretion by actin cytoskeleton remodelling: role of Gelsolin and cooperation with the MAPK signalling pathway. J Cell Sci 119:2156–2167

Valenti R, Huber V, Filipazzi P et al (2006) Human tumor-released microvesicles promote the differentiation of myeloid cells with transforming growth factor-beta-mediated suppressive activity on T lymphocytes. Cancer Res 66:9290–9298

Vandendries ER, Furie BC, Furie B (2004) Role of P-selectin and PSGL-1 in coagulation and thrombosis. Thromb Haemost 92:459–466

van Doormaal FF, Kleinjan A, Di Nisio M (2009) Cell-derived microvesicles and cancer. Neth J Med 67:266–273

VanWijk MJ, VanBavel E, Sturk A et al (2003) Microparticles in cardiovascular diseases. Cardiovasc Res 59:277–287

Whitlow MB, Klein LM (1997) Response of SCC-12F, a human squamous cell carcinoma cell line, to complement attack. J Invest Dermatol 109:39–45

Wolf P (1967) The nature and significance of platelet products in human plasma. Br J Haematol 13:269–288

Wysoczynski M, Ratajczak MZ (2009) Lung cancer secreted microvesicles: underappreciated modulators of microrenvironment in expanding tumors. Int J Cancer 125:1595–1603

Yingling JM, Blanchard KL, Sawyer JS (2004) Development of TGF-beta signalling inhibitors for cancer therapy. Nat Rev Drug Discov 3:1011–1022

Zwaal RF, Bevers EM (1983) Platelet phospholipid asymmetry and its significance in hemostasis. Subcell Biochem 9:299–334

Zwicker JI, Liebman HA, Neuberg D et al (2009) Tumor-derived tissue factor-bearing microparticles are associated with venous thromboembolic events in malignancy. Clin Cancer Res 15:6830–6840