Microvesicles and cancer

M-A. Azerad, F. Debaugnies, A. Demulder, D. Bron, A. Efira

Microvesicles (MV) are since quite recently recognized as forming a unique network between cells. These very little fragments (<1 µm size) are actively released from their parent cells and are able to transfer both cellular and nuclear material. Although active debate remains on how to best detect MV, rendering some results questionable, high MV levels have been reported in aggressive tumours and have been correlated with a poor clinical outcome. Some tumour cell derived MV exhibit strong tissue factor dependent procoagulant activity. Their detection could actually predict the thrombotic risk in selected cancer patients. A growing body of evidence suggests cell microvesicles to be a major link between cancer and thrombosis. Current knowledge on MV in cancer will be reviewed here.
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Introduction

There is active debate in the literature on a definition of microparticles (MP) and microvesicles (MV). The basic mechanism of MP formation is disruption of the machinery supporting asymmetry of phospholipids between the two layers of the membrane. Platelet-derived microparticles (PMP) constitute the majority of the pool of MP circulating in the blood. Besides cancer, high levels of MP have been demonstrated in inflammatory diseases, renal insufficiency, diabetes, heart diseases.[1]

Current definition of MP is based on size parameters: small plasma membrane vesicles (<1 μm) shed from the outer membrane of the cells upon their activation or apoptosis.[1] They carry the epitopes of the cells they are issued from.

Exosomes are smaller microvesicles (30-80nm) which can also be actively released and contain both cellular and nuclear material. Exosomes are formed within endosomal structures, then released and exhibit different markers as compared to MP. Although this classification based on the different origin (intracytoplasmic bodies) and molecular content is obvious for many authors, others consider that the distinction between MP and exosomes is probably not justified since the same mechanism of active transfer of materials does exist for both types of structures.[2,3] MV act indeed as a real network and allow at a nano-level a highly potent communication system between cells.[4]

Cancer has long been associated with thrombosis. MP have been shown to carry Tissue Factor (TF) which can be activated at the site of vascular injury or bind to activated platelets initiating then locally thrombin generation, thereby activating coagulation.[5]

Tesselaer et al in 2007 published a revolutionary paper stating that they found the 'missing link between thrombosis and cancer'.[6]

Other papers have confirmed that elevated levels of MP can be found in different tumour types: glioblastomas, prostate, pancreas, colon, head and neck and breast cancer, high levels have been associated with a poor prognosis.[3]

As shown in *figure 1,* MV can facilitate cancer progression via different pathways.

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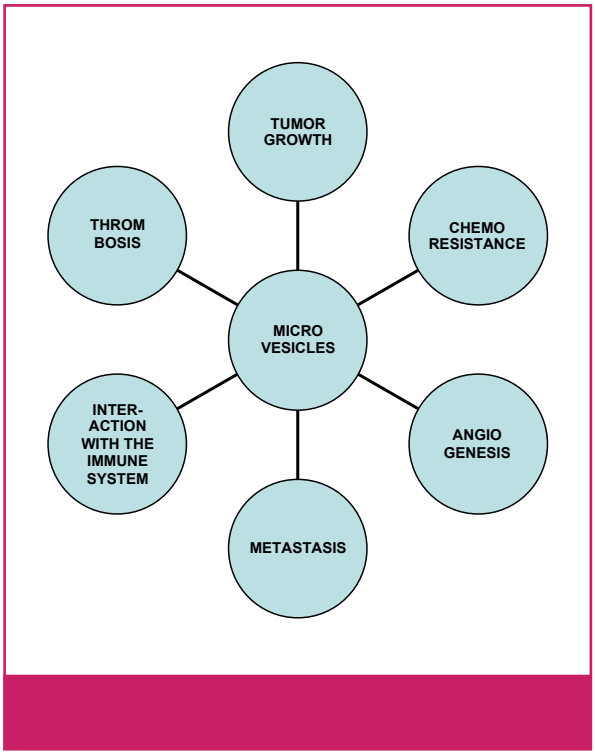

Figure 1. MV can facilitate cancer progression via different pathways.

In this review we will describe the current knowledge on the role of MV (both MP and exosomes) in cancer development and focus on the prospects of this tremendous network.

How to detect microparticles

MP determination remains a real issue. First evidence of microparticle existence comes from electronic microscopy. Later and long considered as gold standard, flow cytometry allowed identifying the origin of the MV: platelet, endothelial, leucocytes, erythrocyte or tumour cell. However some problems do remain: lack of standardisation and detection limits, since standard instruments cannot detect fragments under 300 nm in size. Indeed, more attention is given to even smaller particles less than 100 nm in size, considered at least as important as their bigger siblings. New techniques are able to detect fragments less than 0,3 μm in size but require an experimented staff. Intense research is ongoing in this domain.[7] The ISTH (International Society of Thrombosis and Haemostasis) Scientific and Standardization Committee on vascular biology underlined the need for standardisation of the pre-analytical conditions used in the determination of MPs and has launched a standardisation project for MV analysis. Indeed sampling, centrifugation, freezing and methods, have a great influence on these measurements making comparisons between studies difficult. Once collected, blood should be processed within two hours to avoid platelet activation and MV release. Collection via central venous lines by itself could trigger elevation of MP only due to the sampling procedure. The favourite protocol currently includes collection of blood on citrate and centrifugation twice at 2500g for fifteen minutes before snap-freezing at -80°C (ISTH congress 2011 standardization committee, oral communication). Impedance based flow cytometer with smaller gates has been used but is prone to blockage. ELISA assays do not capture all the vesicles and detect soluble antigens as well. Dynamic light scattering and atomic force microscopy used in research have shown that flow cytometry only reveals a very little part of the circulating MP.[8] Functional methods have been developed and allow to detect the activity of phospholipid and/or tissue factor bound MP in a simple and reproducible way through thrombin generation but this technique does not allow the phenotypic determination of the MV.[9] Quite recently, fluorescence Nanoparticle Tracking Analysis (NTA) has been described and seems quite promising. With this method, vesicles are visualised by light scattering using a light microscope. A video is taken, and the NTA software tracks the Brownian motion of individual vesicles and calculates their size and total concentration. NTA can measure cellular vesicles as small as 50 nm and is more sensitive than conventional flow cytometry (lower limit 300 nm). By combining NTA with fluorescence measurement, vesicles can be labelled with specific antibody-conjugated quantum dots, allowing their phenotype to be determined.[10] Several recent studies suggest that activation of coagulation, perhaps mediated by tissue factor rich MP (TF-MPs) is linked to oncogene induced malignant transformation. Occurrence of deep venous thrombosis either before or concurrent with the diagnosis of cancer appears to predict an aggressive behaviour and correlates with increased tumour angiogenesis and early onset of distant metastasis.[11]

Relationship between microparticles and thrombosis?

Cancer patients most often present an increased risk of thrombosis but also of severe haemorrhage.[12]

In essential thrombocythemia (ET), this can be a real challenge.

Marijke C. Trappenburg and co-workers have shown that patients with ET had higher numbers of circulating microparticles with platelet and endothelial markers, suggesting ongoing platelet and endothelial activation. Microparticles from ET patients are associated with increased thrombin generation, shortened lag time and increased peak height. CD41/CD62E-positive microparticles are elevated only in ET patients with risk factors for thrombosis. These findings suggest a role for microparticles in thrombosis in ET and this deserves further prospective studies.[13]

MV-associated TF is released from tumour cells and can activate coagulation in vitro and in vivo.[14] Can the determination of MV level be yet considered as a simple tool to better assess the coagu- lation risk in our cancer patients? Studies remain contradictory: Zwicker et al have shown, using impedance-based flow cytometry, that elevated levels of microparticles can be found in advanced pancreatic cancer patients and may predict the occurrence of a thrombotic event.[15] More recently, Thaler et al presented the results of a large prospective study, the CATS study following 796 cancer patients during two years. The authors showed a significant higher level of MP in cancer patients versus normal subjects but could not find a correlation between higher MP level and risk of symptomatic thrombosis in this cohort of patients. However, population of cancer patients was not homogenous, while in the work of Zwicker et al only patients with advanced pancreatic tumours were included. The technique used was also different. The measurement of MPs was performed after capture onto immobilised annexin V, and determination of their procoagulant activity was quantified with a prothrombinase assay.[16] Based on his former observation, Zwicker et al started the microTEC study to evaluate the benefit of primary prophylaxis in advanced cancer patients with increased levels of circulating tissue factor bearing microparticles.[17]

In multiple myeloma, thrombosis occurs frequently at baseline and following therapy.

Auwerda et al showed that the levels of TF-MP activity was higher in 122 untreated myeloma patients than in controls but could find no correlation between the level of MP-TF activity before starting treatment and further development of thrombosis. However patients were randomised to three induction treatment groups and ultra-centrifugation was used. This could explain a reduction in MP number.[18]

Role of microvesicles in tumour growth?

In haematological disorders, Ghosh et al have recently shown that circulating microvesicles in B-cell chronic lymphocytic leukemia (CLL) can stimulate marrow stromal cells.

In B CLL, microvesicles have been associated with a more aggressive behaviour of the disease. A shift of MV origin from platelet to leukemic B cell was observed during progression.[19] There are several other examples in the literature where MV influence the microenvironment and promote tumour growth, in glioblastoma or prostatic cancer cell lines.[20,21]

Castellana et al highlighted the intercellular cross-talk between tumour and fibroblasts through MV in prostatic cancer cell lines. TMV induce activation of fibroblasts increased mobility and resistance to apoptosis and also promoted MV shedding from fibroblasts.[21]

MV can also help cells to escape from apoptosis by releasing caspase 3-containing MV, preventing its intracellular accumulation. Hussein et al described that cells indeed accumulate caspase 3 and undergo apoptosis when microvesicles release is inhibited.[22]

Microvesicles facilitate metastasis

Adherence of cancer cells to the vessel wall

The procoagulant properties of cancer cell-derived MV may further support intravascular fibrin formation, which will facilitate adherence of cancer cells to the vessel wall.[3]

Local invasion

MV are also likely to enhance tumour expansion by extracellular matrix degradation, Janowska-Wieczorek et al have shown that platelets derived microvesicles stimulate the production of metalloproteases by breast cancer cell lines. These matrix metalloproteinase (MMP-2 and MMP-9) degrade basement membrane collagens, allowing environmental degradation and tumour dissemination.[23]

Intercellular transfer of oncogenes via microvesicles

Detection of micro RNA expression in human peripheral blood vesicles has been demonstrated.[24]

In glioblastoma, cancer cell-derived MV contribute to horizontal intercellular transfer of the truncated

oncogenic form of the epidermal growth factor receptor (EGFRvIII) from glioma cancer cells to glioma cells lacking this receptor. The recipient cells become transformed and exhibit characteristic EGFRvIII-dependent changes in expression levels of target genes.[20,25]

Microvesicles can induce chemoresistance

Drug resistance is a major cause of cancer treatment failure. Jaiswal et al demonstrated that MP isolated from leukaemia and breast cancer cell lines were cocultured with their drug sensitive counterparts are able to confer multidrug resistance (MDR).[26]

Earlier on, Shedden et al and Safaei and co-workers observed that chemo-insensitive cancer cell lines express more membrane shedding-related genes compared with chemo-sensitive cells. Furthermore, the microvesicles contained high levels of the chemotherapeutic agent doxorubicin or cisplatin.[27,28]

Microvesicles can promote angiogenesis

MV possess a therapeutic potential regarding angiogenesis. They can interact directly through interaction with the ligand receptor. They can also modulate soluble factor production involved in endothelial cell differentiation, proliferation migration and adhesion. MV are able to reprogram endothelial mature cells and to induce changes of endothelial progenitor cells.[29] It has been recently shown that microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niches.[30]

The procoagulant effect of microvesicles also indirectly leads to the release of growth factors. Thrombin activates cells via cleavage of protease-activated receptors (PARs), and this activation results in the release of vascular endothelial growth factor (VEGF).[31]

Microvesicles and the immune system

Pap recently reviewed the role of microvesicles in malignancies and *figure 2* (issued from her recent paper with kind authorisation) summarises the roles of MV in tumorigenesis.[32]

Cancer cells escape complement induced lysis through release of vesicles containing the complement inhibitor membrane cofactor protein CD46. CD46 promotes inactivation of complement C4b and C3b.[33,34] MV from various cancer cells expose Fas ligand (FasL, CD95L), a ligand of the FAS receptor (CD95) and /or TRAIL. This induces T-cell apoptosis and impairs the function of adaptive immune cells.[3] After stem cell transplantation, elevated levels of endothelial derived MP have been observed in the early phase after transplantation (two to three weeks) and were related, with both an elevation of soluble Fas Ligand and GVHD after allogenic graft.[35] Cancer cell-derived microvesicles are able to fuse with plasma membranes of monocytes, thereby impairing their differentiation to antigen-presenting cells.[36] Another way to escape the immune surveillance has been suggested by Janowska-Wieczorek A et al. Platelet MV isolated from outdated platelets transferred platelets derived integrin CD41 to the surface of breast cancer cell lines. This might allow cancer cells to hide from the immune system.[37] In acute myeloid leukaemia (AML), Szczepanski MJ et al have provided evidence that MV present in sera of patients with newly diagnosed AML play a role in regulating NK activity. They showed also that IL-15 is able to counteract immunosuppressive effects mediated by TGF-carried on microvesicles from AML patients. This strengthened the potential of IL-15 for AML therapy.[38]

Therapeutic prospects

Some chemotherapy like placitaxel, Vinca alcaloids, besides their antitumoural potential, interfere with microvesicle and exosome release.[3,31] Immunotherapy using autologous dendritic cell-derived microvesicles is currently tested in phase II clinical trials in melanoma and non small cell cancer patients with promising results.[39,40]

Conclusion

Cancer cell derived microvesicles do exist and play an important role in tumour progression.

Ongoing prospective studies will assess if tissue factor bearing microvesicles levels can be a reliable marker of thrombotic risk.

It also leads the path to a new therapeutic domain, where well designed trials are already ongoing in selected tumour types.

Once standardised, the presence of MV may be a useful marker for tumour bulk and overall survival in cancer patients. They could also allow monitoring the efficacy of the cancer treatment.

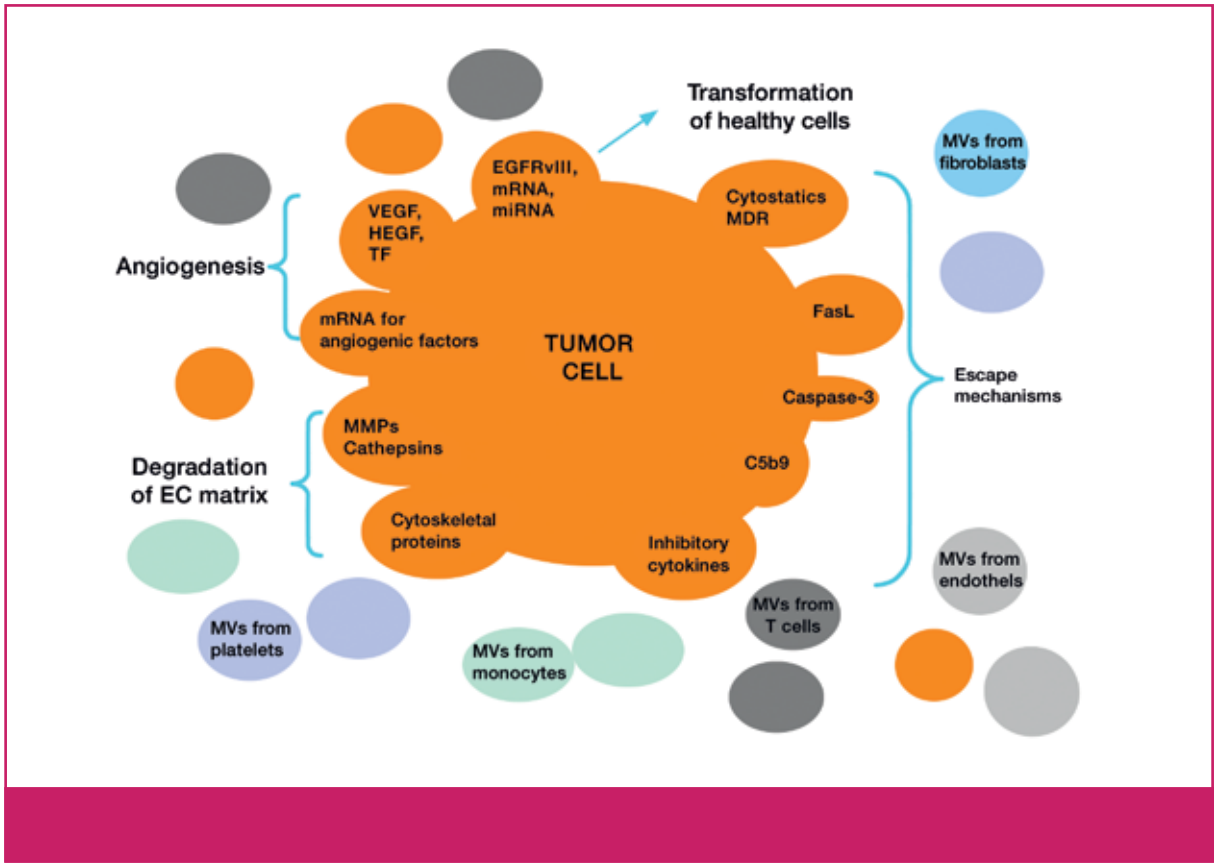

Figure 2. Roles of MV in tumorigenesis. With permission from Pap (Adv Exp Med Biol 2011).

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Key messages for clinical practice

Microvesicles promote communication between cells via a novel pathway and also offer a fascinating link between coagulation and cancer. Their role in tumour progression and multidrug resistance is now being actively studied. Different techniques are used for their detection with currently no gold standard method. Microvesicles could be used in the near future as prognostic markers in cancer and also as a therapeutic tool.

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