New methodological strategies in haematology using cell-derived microvesicles

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Revista de Hematología

Rev Hematol Mex 2013;14 (Supl. 1):S38-S41

Programa educativo

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Microvesicles as messengers of cell and tissue dysfunction

Cellular microvesicles are membrane nanometric vesicles, 0.1-1 μm in size, released into body fluids by activated cells or during apoptosis in a variety of pathological conditions [1-4]. Characteristically, cell activation disturbs phospholipids transport and cytoskeleton membrane connexions resulting in phosphatidylserine exposure, membrane blebbing and vesiculation (Fig. 1).

The most well known cellular MVs are those of platelet, leukocyte, erythrocyte and endothelial cell origin found in circulating blood [5]. A number of studies have demonstrated that stimulation of these cells is followed by the characteristic features of cell activation: increased levels of cytoplasmic calcium associated to translocation of phosphatidylserine from the inner to the outer leaflet of the membrane and activation of calpains that, by cleaving cytoskeletal filaments, facilitate MVs shedding [6]. The increase in intracellular Ca^{2+} concentration induces a disordered state in the phospholipids asymmetry of quiescent cells that is normally maintained by the concerted activity of transporter proteins [7, 8]: the ATP-dependent inward- and outward-directed transporters, flippases (aminophospholipid translocase) and floppases (including the ATP-binding cassette transporter ABCA1) respectively, and the Ca^{2+}-dependent scramblases that facilitate bidirectional movement between the 2 membrane leaflets. The rate of phosphatidylserine translocation has been found to be sensitive to the altered expression of ABCA1 [9].

Microvesicles reflect the state of the parent cell

Besides phosphatidylserine microvesicles convey surface identity antigens and contain biomolecules that allow their identification and functional characterization [10]. These membrane glycroproteins and cytoplasmic components include the coagulation trigger tissue factor (TF), fibrinolytic enzymes, growth factors and their receptors, inflammatory mediators (cytokines, chemokines) and even mRNA or miRNA. Microvesicles may thus be considered as messengers of cell and tissue damage [10] and their presence in the circulation and other body fluids constitutes a signature of cell activity or dysfunction [11]. For these reasons, MVs have been proposed as pathogenic markers, key players of the haemostatic response [12]. At present, the most solidly established applied research on MVs is their procoagulant activity as a determinant of thrombosis risk in various clinical conditions [4, 13-15]. Evidence obtained is however largely associational and the main clinical application of MVs analysis has been the simple correlation of their levels with various disease states including cardiovascular diseases, diabetes, autoimmune diseases, inflammatory processes, sepsis and cancer.

Vascular ischemic accidents are the result of an occlusive thrombus formed in situ or of an embolus. Although a defect in fibrinolysis and/or localised proteolysis [16] is certainly implicated, we do not have at present a reliable methodology to accurately assess the fibrinolytic activity in the intravascular space. Recent data strongly

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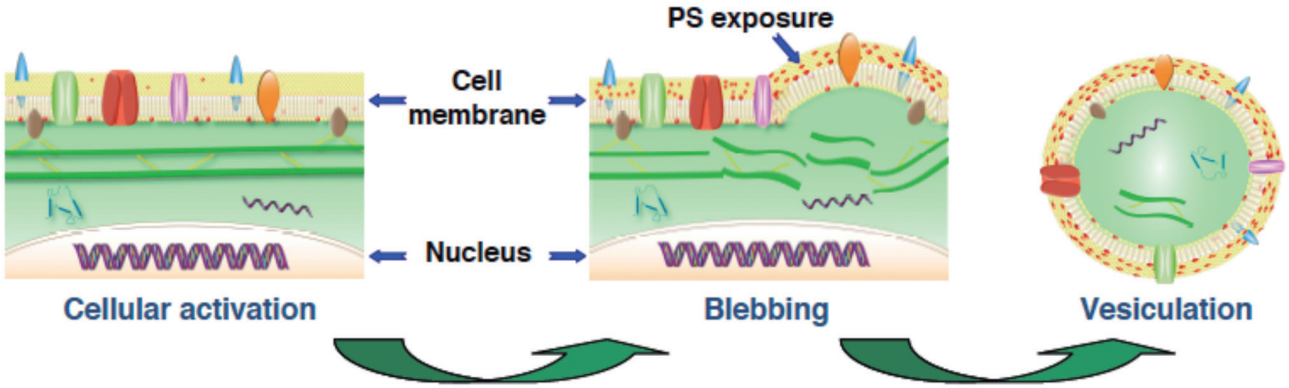

Figure 1. Scheme of cell activation, phosphatidylserine (PS) exposure, membrane blebbing and release of microvesicles (vesiculation).

suggest that cellular MVs may be an important source of fibrinolytic and proteolytic activity in circulating blood [17]. For instance, MVs bearing plasminogen activators transform plasminogen into plasmin at their membrane and participate in a new fibrinolytic cross-talk mechanism that was only recently evidenced [18]. (see Angles-Cano E & Plawinski A, corresponding chapter in this volume).

Microvesicles: a dynamic pool of bioactive effectors

Other than the procoagulant factors and their distinctive glycoproteins, MVs may also carry bioactive components (membrane receptors, cytokines, transcription factors, mRNA) that are veritable indicators of the state of activation of the parental cell, thus constituting a disseminated dynamic pool of bioactive effectors or messengers, as documented by several *in vitro* studies [17 25, 19 22, 20 17]. Some of these MV components may exert local functions or be transferred to other cells: local fibrinolytic and proteolytic activities induced by uPA and metalloproteinases; transfert of GPIIb/IIIa from platelet MVs to leukocytes; transfert of mRNA, transfert of chemokin receptor; transfert of TF from leukocytes MVs to platelets, delivery of infectious agents into cells (human immuno deficiency virus, prions) [21] and transfert of oncogens from glioma MVs to naïve cells. Using proteomic approaches (two-dimensional electrophoresis and mass spectrophotometry), the number of proteins being identified in MVs of several origins has importantly expanded [22 24, 23 36, 24 34].

Microparticle's identity unveil activated or suffering cells

Membrane glycoproteins distinctive of the parental cells are present on circulating MVs allowing thereby identification of their cellular origin. Antibodies directed against these cell-specific antigenic determinants are used for this purpose in flow cytometry or antibody capture assays. An increase in the number of distinct MVs is now considered as an indicator of platelet, endothelial or leucocyte activation [25 42, 26 16].

Identification of MVs constitutes therefore a solid advantage to determination of their sole number and represents a robust parameter when associated to thrombotic, systemic or inflammatory diseases. Furthermore, identification of MVs of practically any cell origin in plasma or other biological fluids (cerebrospinal fluid –CSF-, tears, exudates etc) would become possible if antibodies directed against cell-specific antigenic determinants were available. Their detection would certainly be considered as a direct message of tissue specific activation or damage. For instance, the hypothesis that tumour-derived TF-positive MVs in plasma contribute to cancer-associated thrombosis is based on the finding of these MVs in patients with solid tumours and venous thrombosis. Clotting tests using tumour cell samples suggest that cancer cells are a potential source of circulating TF-positive MVs [27]. More specific information could be obtained if MVs of tumour origin (solid or leukemic) could be used as early messenger of relapse.

In recent years, tumour MVs have evolved as potential biomarkers. Indeed, tumour cells are able to constitutively release large amounts of MVs bearing tumour specic antigens [28]. These MVs may found into the bloodstream and other bodily fluids. Microvesicles released by malignant cancer cells can transfer various messages to target cells and may be critical to disease progression [29]. For example, solid tumours that are difficult to reach and de-

tect may reveal their presence by releasing MVs, and the presence of tumour-derived MVs in biological fluids may also be useful for detecting metastases.

In hematologic malignancies the study of MVs is gaining increased interest. For instance B-Cell-derived MVs from chronic lymphocytic leukaemia (CLL) express separate phenotypes during leukemic disease progression and underscores the important role of MVs in activation of the tumour microenvironment [30]. TF-bearing promyelocytic-derived MVs in acute promyelocytic leukemia have been identified using and antibody to CD33. These MVs decreased the coagulation time and induced thrombin generation, thus indicating that the procoagulant state in acute promyelocytic leukemia is partially due to the TF-dependent procoagulant properties of circulating promyelocytic-derived MVs [31, 32]. Furthermore, procoagulant myeloblast-derived MVs were recently described in acute myeloblastic leukaemia [33]. Myeloblas origin was defined by cytofluorimetry using antibodies to CD117, CD13 and CD34. These MVs were highly procoagulant as determined with a thrombin generation test. The release of MVs by mature B cell tumours in childhook leukaemias may be related to the cellular activation status or to the activity of the leukaemia cell type [34].

In a proteomic study of mature B-cell neoplasms with B-cell hyperlymphocytosis, including CLL, small cell lymphoma from hairy cell leukemia or splenic lymphoma with villous lymphocytes, CD148 was identified on lymphocyte MVs. The presence of this marker in MVs excludes the diagnosis of CLL and allows mantle cell lymphoma diagnosis to be suspected [35].

In summary, the generation of leukaemia/lymphoma cell MVs constitute a new tool for diagnosis and clinical/therapeutic follow-up.

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