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Extracellular vesicles as new players in angiogenesis

Sharad Kholia, Andrea Ranghino, Paolo Garnieri, Tatiana Lopatina, Maria Chiara Deregibus, Pietro Rispoli, Maria Felice Brizzi, Giovanni Camussi

Abstract

Growing evidence suggests that small vesicles actively released from cells may encapsulate transcriptional regulators and RNA molecules. Their ability to interact with neighbouring cells and/or with distant cells through biological fluids, makes them a medium through which intercellular exchange of information can happen. Recently, membrane vesicles, which include exosomes and microvesicles, gained a place amongst the vast group of angiogenic mediators. In the present review we discuss the potential relevance of these vesicles in physiological and pathological situations of angiogenesis as well as their mechanism of action.

Abbreviations

EVs, extracellular vesicles; MVBs, multivesicular bodies; ESCRT, exosomal sorting complex required for transport; DCs, dendritic cells; lncRNA, long noncoding RNA; miRNA, microRNA; MMP, matrix metalloproteinases; EGFRvIII, epidermal growth factor receptor vIII; VEGF, vascular endothelial factor; VEGF-R2, vascular endothelial factor-receptor 2; FGF, fibroblast growth factor; EGF-R, epidermal growth factorreceptor; IL-3, interleukin-3; IL-6, interleukin-6; IL-8, interleukin-8; TIMP-1 and -2, tissue inhibitors of metallo-proteinases-1 and -2; ECFCs, endothelial colony-forming cells; NOS, nitric oxide synthase; ASCs, mesenchymal stromal cells from adipose tissue; PDGF, platelet derived growth factor; SCF, stem cell factor; MSCs, mesenchymal stromal cells; MCP-1, monocyte chemoattractant protein-1; SCID, severe combined immune deficiency; HMEC, human microvascular endothelial cells.

1. Introduction

Angiogenesis is a tightly regulated process that is essential not only throughout embryo development, but also during the maintenance of vascular homeostasis in adult organisms. Moreover, new vessel formation is also critical in several processes involving tissue regeneration. Angiogenesis is normally initiated after preliminary destabilization of pre-existing vessels, whereby, the proliferation and migration of endothelial cells cause endothelial sprouting, canalization and eventually stabilization of the vessel wall leading to the formation of new blood vessels [1]. Complex arrays of soluble factors together with cell-to-cell and cell-to-matrix interactions modulate angiogenesis at a physiological level. However, an altered form of angiogenesis may occur in several pathological conditions including tumours as well as in various inflammatory states whereby the process may be dysregulated leading to an abnormally enhanced or reduced level of new vessel formation.

Recently, vesicles released by cells have also been implicated in the array of mediators involved in angiogenesis. The phenomenon of vesiculation has been for long time considered a hallmark of cell injury and vesicles present in biological fluids or in culture medium were retained as fragments of dying cells. More recently, it has been discovered that perfectly healthy cells also release vesicles and exploit them as vehicles for sharing biologically active molecules with other cells in a paracrine and/or endocrine fashion [2]. This new mechanism of cell-to-cell communication relies on vesicle mediated transfer of bio-active lipids, proteins, receptors and nucleic acids. In particular, the transfer of transcriptional regulators and non-coding RNAs may account for the epigenetic changes induced by these vesicles in recipient cells. Interestingly, communications mediated by vesicles have emerged to be a well preserved evolutionary mechanism in the three life kingdoms of protists, plants and fungi [3], [4], [5], [6], [7] as well as in invertebrates [8] and vertebrates [9]. In mammalians this mechanism of inter-cellular communication has been involved in multiple biological, physiological, and pathological processes including angiogenesis [10].

2. Extracellular vesicle biogenesis and function

Two main classes of non-apoptotic vesicles released by cells have been identified [2]. Firstly, exosomes originate from invaginations of endosomal membranes of multivesicular bodies (MVBs) which fuse with the plasma-membrane leading to exosome release. Secondly, vesicles formed on the cell surface through budding of the plasma-membrane ensuing in extracellular release. For this latter class of vesicles, the literature has attributed

various names including: ectosomes, shedding vesicles, microvesicles and microparticles. Moreover, they have also been entitled on the basis of their function or tissue of origin for example: tolerosomes, cardiosomes, and prostatosomes [11], [12], [13]. Despite the divergence in the formation of these two classes of vesicles, they have similar functions, similar mechanisms of membrane budding as well as several overlapping characteristics. Furthermore, cells frequently release them concomitantly and therefore it has been proposed to collectively identify them as “extracellular vesicles” (EVs) [11].

The assembly of EVs depends on the accumulation of their constituents in small membrane domains that bud into MVBs to form exosomes, or on cell surface to form shedding vesicles. These small membrane domains are assembled from several lipids such as cholesterol, phospholipids, sphingomyelins, ceramide and proteins [12], [13], [14], [15]. One such protein is Alix, an accessory protein of the endosomal sorting complex required for transport (ESCRT) which has been implicated in the transfer of proteins to these small membrane domains [13]. Furthermore, due to its multiple protein-binding sites, Alix has also been associated with multifunctional activities in exosome biogenesis [14]. ESCRT, originally considered specific for exosome biogenesis, has also been recently identified to participate in the assembly and release of vesicles shed through plasma-membrane budding [13]. Furthermore, biogenesis of shed vesicles requires in addition, binding to plasma-membrane anchors as well as high-ordered polymerization of specific cytoplasmic proteins which do not interact with MVB membranes during exosome assembly [16]. Nevertheless, the molecular contents of exosomes and shedding microvesicles are similar and include heat-shock proteins, cytoskeleton proteins and several cell specific proteins and RNA species [17]. Moreover, the EV plasma membrane frequently expresses molecules and receptors representative of the cell of origin.

The functional role of EVs in intercellular communication is dependent on their interaction with cells present in the surrounding extracellular milieu. Released EVs may interact with the originator cells therefore acting as autocrine mediators and with other cell types thus acting as paracrine/endocrine mediators. Furthermore, the interaction can be mediated either by direct fusion or through specific receptors leading to the fusion of EVs with the recipient cell's plasma-membrane causing release of their contents intracellularly. The mechanism of uptake may vary depending on the origin of EVs as well as on the target cells. We found for instance that EVs derived from proangiogenic progenitors express mainly L-selectin for the interaction with endothelial cells[18], whereas those derived

from mesenchymal stem cells mainly express integrins and CD44 that enhance their binding to epithelial cells [19]. Furthermore, the uptake of EVs from the placenta was due to the expression of syncytin-1, which belongs to a family of mammalian fusogens [20]. Studies based on lipid fusion assay have also shown that EV uptake by dendritic cells(DCs) depends on fusion of cholesterol rich plasma membrane microdomains as well as cytoskeleton activation [21], [22]. An alternative mechanism by which EVs can deliver their content to target cells is through receptor-mediated [23] or receptor-independent clathrin-mediated endocytosis and micropinocytosis [24]. Once fused or internalized, EVs release their biologically active contents rich in proteins [25], [26], or oncogenic products [27] that can induce functional changes in recipient cells. Moreover, EVs also carry transcription factors and nucleic acids such as mRNA, long non-coding RNA (lncRNA) and microRNA (miRNA) [28], [29], [30] that could induce transient or persistent epigenetic modifications in recipient cells. This form of transport of active molecules by EVs is advantageous as the encapsulation provides protection from degrading enzymes in the microenvironment. Finally, EVs may also act as a signalling-complex without the need of internalization. An example of this mechanism was provided by Raposo et al. [31], who demonstrated that vesicles released from B lymphocytes could activate T cells by presenting the MHC-II-peptide complex expressed on their surface.

3. EV modulation of angiogenesis

Most of the studies investigating the angiogenic properties of EVs have been performed on tumour released vesicles. Kim et al. [32] for instance, showed that sphingomyelin expressed on tumour EVs stimulated endothelial cell migration and angiogenesis. Moreover, tumour-derived EVs, rich in matrix metalloproteinases (MMP) [33] as well as in the extracellular MMP inducer CD147 [34], could have a role in the degradation of extracellular matrix proteinsnecessary for the angiogenic process.

The EV membrane is enriched in tetraspanins such as Tspan8, that together with other adhesion molecules, has been linked with the endothelial uptake of vesicles [35]. As a result, an upregulated expression of VEGF, VEGF-R2 and von Willebrand factor (vWF) has been observed in recipient endothelial cells [36], eventually leading to the stimulation of vessel sprouting and branching [37]. Furthermore, the mutated oncogenic epidermal growth factor receptor (EGFRvIII) carried by EVs released by glioma cells was recently attributed to enhance the production of VEGF and VEGF-R2 through the activation of MAPKand Akt pathways in endothelial cells [27]. In addition, it has also been reported

that tumour-derived EVs may contain and deliver not only various growth factors, pro-angiogenic modulatory proteins such as VEGF, FGF, EGF-R, IL-6, IL-8, and angiogenin, and anti-angiogenic proteins like TIMP-1 and -2, but also nucleic acids such as mRNAs which may be translated into active proteins within recipient cells [38].

Interestingly, we have also found that EVs with proangiogenic properties can be released by a subset of renal cancer stem cells defined on the basis that: they were positive for the expression of stem cell markers such Nestin, Nanog and Oct3–4; they exhibited the ability to induce serially transplantable tumours starting from a number of cells as low as 100 cells; and they had the capability to generate clones and to grow in spheres [26]. These vesicles were found to be enriched with pro-angiogenic mRNA, miRNA and proteins (MMP2/9, angiopoietin 1, ephrin A3, FGF and VEGF) that could favour tumour vascularization and lung metastasis by priming endothelial cells [26].

Hypoxia is a factor that favours the accumulation of pro-angiogenic molecules in tumour derived EVs [39]. It has been shown for instance that hypoxia enhances the compartmentalization of pro-angiogenic miRNAs such as the miR-210 in tumour EVs [40], or miR-126 and miR-296 in EVs derived from proangiogenic progenitors [41].

Proangiogenic progenitors are bone marrow derived cells present in the circulation [42] that have been implicated in different angiogenic conditions such as tissue healing, cancer and inflammation. These cells have been shown to exist in two different populations as described by Yoder et al. [43]. One population also known as proangiogenic progenitors or as endothelial colony-forming cells (ECFCs) expresses endothelial markers such as vWF, KDR, CD31, CD146, CD105, CD144 and UEA-1 but not haematopoietic markers such as CD45 and CD14. The other population is considered to be of myeloid progenitor origin and expresses the haematopoietic markers CD45 and CD14.

During an injury, proangiogenic progenitors accumulate at the affected site and act mainly in a paracrine manner releasing cytokines, growth factors, as well as EVs. For instance, we found that these cells released EVs that induced neo-angiogenesis both in vitro and in vivo through horizontal transfer of functional mRNA to quiescent endothelial cells [29]. The transfer of mRNA and its translation into protein was confirmed through the transduction of GFP protein in endothelial cells that were exposed to proangiogenic progenitor derived-EVs containing GFP-mRNA. Moreover, the biological activity demonstrated by the EVs was significantly reduced when inactivating their RNA content. Subsequent studies further

revealed the presence of miRNAs as well in EVs derived from proangiogenic progenitors which also exhibited angiogenic activity relevant to their function [41]. Taken together these experiments suggest that RNA delivered by EVs contributes towards the angiogenic potential of proangiogenic progenitor-derived vesicles.

Endothelial cells on the other hand, may also release EVs that contribute towards vascular homeostasis through autocrine and/or paracrine activities. It has in fact been shown that endothelial EVs may potentially transfer the protein Delta-like ligand 4 to neighbouring endothelial cells that may promote angiogenesis by blocking the Notch signal [44]. More recently, we have also found that in an inflammatory microenvironment rich in interleukin IL-3, endothelial cell-derived EVs transfer the activated form of the functional transcriptional factor STAT5 to neighbouring cells.

4. EVs in angiogenic tissue regeneration

In the context of tissue regeneration, the concept of stem/progenitor cell EVs exhibiting properties to activate angiogenesis has been investigated in several studies. We demonstrated that EVs derived from proangiogenic progenitors elicit a pro-angiogenic phenotype in quiescent human micro- and macrovascular endothelial cells when internalized [29]. The size of these vesicles as observed by Nanosight analysis and electron microscopy was in the nanometre range of 60–130 nm (Fig. 1A) and they were mainly sorted from the cell surface [41]. Moreover, cytofluorimetric analysis showed that these EVs were positive for endothelial markers such as α4 and β1 integrins, L-selectin, CD105, CD31 and VEGFR1 (Fig. 1B) but not P-selectin, CD42b (platelet markers) and CD14 (monocyte marker).

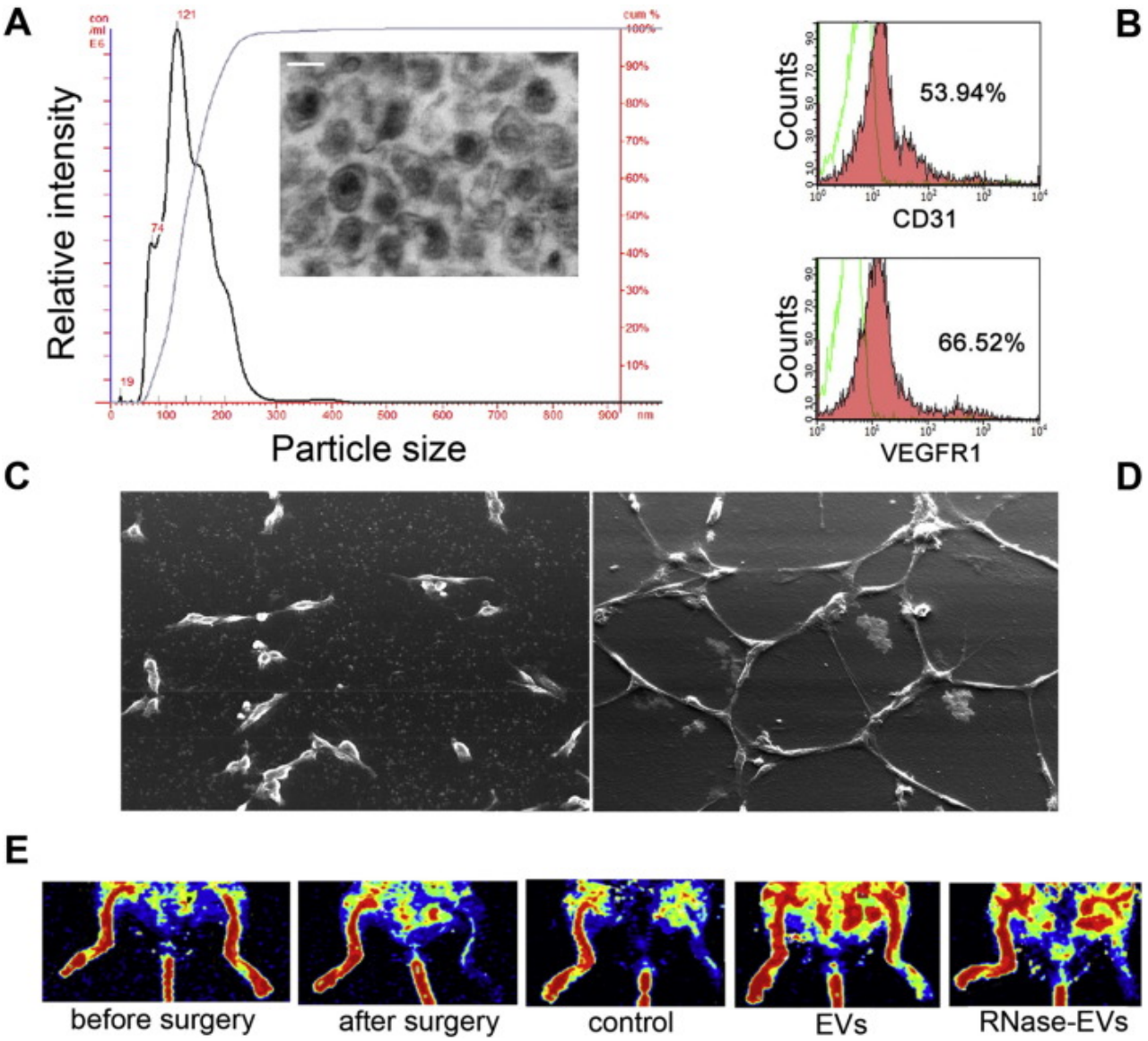

Fig. 1. Angiogenic potential of proangiogenic progenitor-derived EVs. Panel A: nanoparticle tracking analysis of purified proangiogenic progenitor-derived EVs. Inset: representative micrograph of transmission electron microscopy of purified proangiogenic progenitor-derived EVs showing a spheroid shape (white bar = 100 nm). Panel B: representative cytofluorimetric analysis showing the expression of CD31 and VEGFR1 by proangiogenic progenitor-derived EVs. The green lines represent the control isotype antibody. Panel C and D: representative capillary-like structure formation by HMEC plated on Matrigel after 24 hour incubation at 37 °C in the absence (C) or presence (D) of EVs (original magnification, ×500). EPC-derived EVs induced formation of vessel-like structures.Panel E: representative laser doppler analysis of SCID mice with acute hindlimb ischemic injury induced by ligation and resection of the left femoral artery and treated with EVs. The images were obtained before surgery, immediately after surgery and 7 days after surgery in untreated animals (control) and in mice treated with EVs. EV treatment restored perfusion which was absent when RNA in EVs was inactivated by RNase treatment.

Further investigations revealed that α4 and β1 integrins as well as L-selectin present on the EV membrane interacted with endothelial cells and were therefore instrumental for their cellular uptake. Post-internalization, these EVs did not only stimulate endothelial cell proliferation, but also increased their resistance to apoptosis and instigated the formation of capillary-like structures when culturing EV-treated cells on Matrigel [29] (Fig. 1C, D). Moreover, inhibition of these surface molecules (integrins and selectins), with blocking antibodies and proteases, prevented the internalization of EVs as well as the consequential pro-angiogenic effects induced by them in vitro[29]. In vivo, we observed that, injecting severe combined immunodeficient mice (SCID) subcutaneously with EVs incorporated in a Matrigel matrix together with human endothelial cells, favoured the organization of endothelial cells in canalized vessels. Furthermore, as various studies on EV internalization have revealed the transfer of mRNAs related to nitric oxide synthase (NOS) and PI3K/AKT signalling pathways from proangiogenic progenitors to endothelial cells to be critical in the formation of new blood vessels, it can be speculated that the observed effects may be due to the horizontal transfer of RNA carried by EVs from proangiogenic progenitors to endothelial cells [29]. These experiments prompted us to evaluate whether proangiogenic progenitor-derived EVs may prevent and favour recovery from ischemia/reperfusion injury.

In a rat experimental setting of renal ischemia–reperfusion injury, we observed that EVs derived from proangiogenic progenitors, when injected intravenously, were localized within tubular cells and in peritubular capillaries therefore favouring functional and morphological protection of the kidney from acute injury. These EVs not only had a stimulatory effect on the proliferation rate of tubular epithelial cells and increased their resistance to apoptosis but also decreased the infiltration of leukocytes. In addition, they also prevented the development of tubulointerstitial fibrosis, glomerulosclerosis as well as impeded vascular rarefaction [41]. This resulted in the inhibition of the progression of the injury towards chronic renal failure which is normally evident after an acute ischemia/reperfusion injury. The healing effects of EVs observed were mainly attributed to the reprogramming of hypoxic resident renal cells to a more regenerative phenotype through the delivery of RNAs that they are enriched in. In fact, the pro-angiogenic miRNAs: miR-126 and miR-296 carried by EVs were shown to be contributors towards the protective effects observed [41]. Interestingly, reducing the EV miRNA content, either by Dicer knock-down in proangiogenic progenitors or through the use of specific antagomirs together with

inactivating RNAs, by treating vesicles with elevated concentrations of RNases, inactivated the observed EV biological activities [41].

EVs derived from proangiogenic progenitors have been also shown to be effective in the treatment of experimental peripheral artery disease. In fact, EVs from proangiogenic progenitors have been shown to favour neovascularizationin a murine model of severe hind-limb ischemia obtained in SCID mice by ligation and resection of the left femoral artery [45]. Analysis by laser-doppler flowmetry showed not only an increase in hind-limb perfusion but also a reduction in the ischemic damage after EV treatment (Fig. 1E). The contribution of RNAs carried by EVs was investigated by treating mice either with EVs that had RNA inactivated or with EVs released from Dicer knock-down proangiogenic progenitors (in order to achieve miRNA depletion). In these experimental conditions we observed that neovascularization, endothelial repair and regeneration were significantly reduced further confirming the role of RNA in biologically active EVs.

Apart from proangiogenic progenitors, the human mesenchymal stromal cellsfrom adipose tissue (ASCs) have also been reported to release EVs that exhibit a pro-angiogenic potential. Eirin et al. [46] demonstrated that ASC-derived EVs are enriched with several types of RNAs that target gene products that modulate several cellular pathways including angiogenesis. We found in our study that EVs released from ASCs carried several angiogenic factors such as c-kit, MFG-E8, SCF, APRIL, artemin, angiopoietin-like factor and the matrix metalloproteinase MMP-20 which facilitated endothelial cell migration [47]. Furthermore, several pro-angiogenic miRNAs including miR-17, miR-21, miR-126, miR-130a, miR-210 and miR-296 were also discovered to be enriched in these EVs [48]. Interestingly, we also observed that the microenvironment plays an important role in modulation of EV cargo and their biological activity. For example, ASC stimulation with platelet derived growth factor (PDGF), which is involved in the formation of new vessels after damage, not only increased the secretion of EVs but also altered their proteomic content [47] and enhanced angiogenesis in vitro and in vivo (Fig. 2A–C). For instance, expression of some pro-angiogenic molecules that were absent in basal conditions were observed after PDGF stimulation including c-kit, a receptor present in endothelial and haematopoietic progenitor cells, and its ligand SCF [47]. Experiments in which SCF or c-kit was blocked, significantly reduced but not completely abrogated the pro-angiogenic effects of EVs, therefore suggesting that a concomitant action of different mediators is involved. The enhanced expression of MMP2 and MMP9 in EVs derived from PDGF-treated ASCs possibly favours the migration of endothelial cells required for the

formation of new vessels. In fact, the invasiveness of EV-treated endothelial cells was significantly reduced by Batimastat, an inhibitor of matrix metalloproteinases. In contrast, stimulation of ASCs with bFGF induced the release of vesicles which exhibited stabilizing effects on vessel growth [48] (Fig.2D). For instance, several angiogenic proteins expressed in EVs including MFG-E8, artemin, APRIL, angiopoietin-like factor and MMP-20 were down-regulated and expression of angiogenesis modulators such as TRANCE and MMP-7 were upregulated. Furthermore, profiling of mature miRNAs present in EVs revealed the down-regulation of several pro-angiogenic miRNAs after bFGF treatment including miR-194, miR-21, and miR-224 that target TRANCE and miR-210 that targets MMP-7. These vesicles also showed a diverse angiogenic potential: the number of vessels formed by HMEC stimulated with EVs released from bFGF-treated ASCs was significantly lower than in control EVs, but these vessels were larger in size (Fig. 2D). Furthermore, these vessels contained smooth muscle actin positive cells, suggesting formation of more stabilized vessels. Since there was a down-regulation of the pro-angiogenic miR-21 and an up-regulation of the anti-angiogenic miR-223 in EVs from bFGF-treated ASCs, we have also studied their potential role/effects in the bFGF-induced changes of vesicle angiogenic properties. Experiments based on transfection with miR-21 mimic or with miR-223 antagomir suggested that, changes in the balance of the expression of these two miRNAs in vesicles moderate their angiogenic ability. Taken together these data suggest that the microenvironment may influence the ability of EVs to induce new vessel formation by altering their expression of pro- and anti-angiogenic factors [47], [48]. Interestingly, we also studied the potential damaging effects of metabolic environmental cues. In particular we observed that obesity impacts the pro-angiogenic potential of ASC-derived EVs by impairing VEGF, MMP-2 and miR-126 content [49]. This implies that although the observed function of ASC-EVs may make them suitable for use in regenerative medicine, the study of EVs released in different pathological contexts is crucial to assess their proper therapeutic applications.

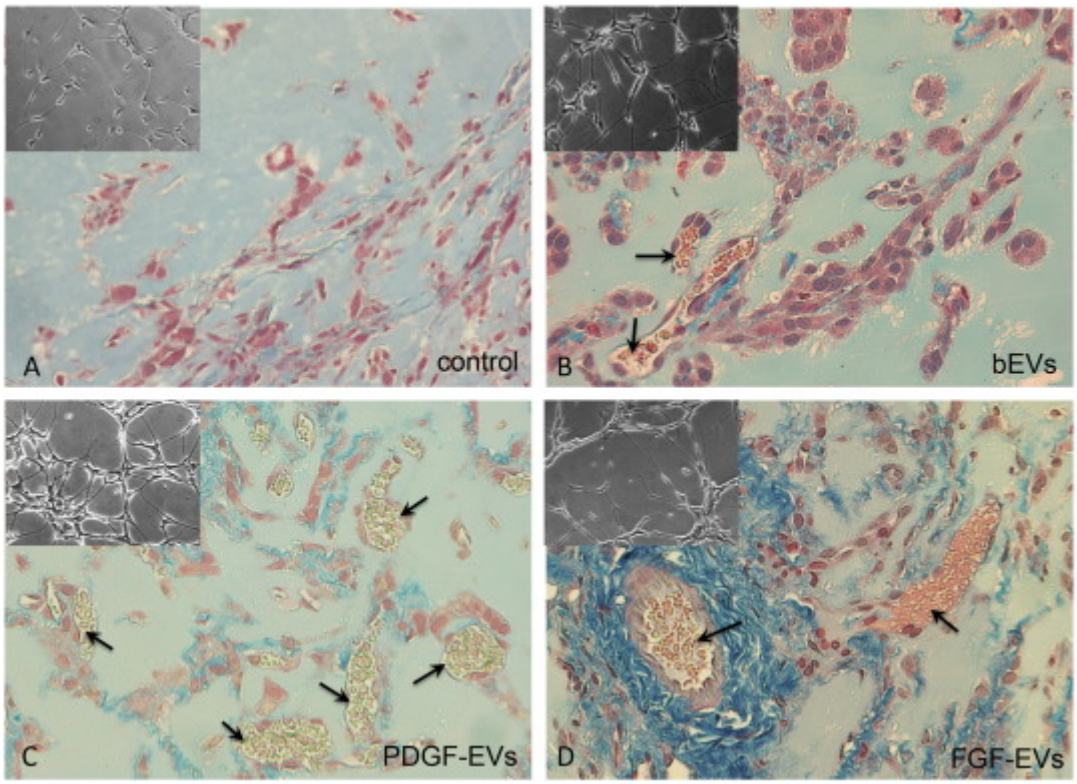

Fig. 2. PDGF and bFGF influence on angiogenic properties of ASC-derived EVs. EVs obtained in basic conditions (b-EV) and EVs obtained after ASC stimulation with PDGF (PDGF-EV) or bFGF (FGF-EV) were used in in vitro and in vivo models of angiogenesis. Representative images of in vivo vessel formation within subcutaneously injected Matrigel containing HMEC (sections stained by trichrome method; arrows indicate red blood cell containing vessels, original magnification ×250); insets show capillary-like structure formation in vitro on Matrigel (phase contrast microscopy, original magnification ×250): (A) non-stimulated HMEC (control), (B) HMEC stimulated with b-EVs, (C) HMEC stimulated with PDGF-EVs, (D) HMEC stimulated with FGF-EVs.

Bone marrow-derived mesenchymal stromal cells (MSCs) were also shown to possess a pro-angiogenic effect. MSCs have been demonstrated to exert cardiovascular protection and to have potential regenerative properties in cardiovascular medicine [50]. The beneficial effect of MSCs in this context has mainly been attributed to paracrine factors and several studies have reported the contribution of EVs as well. For instance, Lai et al. [51] reported that EVs isolated from supernatant of human MSCs reduced the size of the myocardial infarct when infused in a Langendorff mouse heart model of ischemia/reperfusion injury. In a successive experiment, the same group demonstrated that the observed beneficial effects of MSC-derived EVs could be attributed to the enzymes they carry which are essential for the ATP generating stage of glycolysis to increase ATP production, as well as, in the activation of the PI3K/Akt signalling that confers cardioprotection, and a decreased state of oxidative stress, therefore ameliorating

overall cardiac performance [52]. Furthermore, Bian et al. [53] also reported the cardioprotective properties of EVs whereby they showed that EVs released from human bone marrow MSCs, when injected into myocardium in a rat model of acute myocardial infarction, induced migration and proliferation of endothelial cells and promoted angiogenesis in the ischemic area, thereby limiting the size of the infarct [53].

EVs derived from the human umbilical cord MSCs have also recently been implicated to exhibit pro-angiogenic activity. For instance, it has been shown that EVs released by umbilical cord MSCs induced migration, proliferation and angiogenesis in vitro by activating the Wnt/β-catenin pathway [54]. This pro-angiogenic effect was reversed by the β-catenin inhibitor ICG-001 and abrogated by the knockdown of Wnt4. Furthermore, Chen et al. [55]demonstrated that, in hypoxic conditions, umbilical cord-derived MSCs produced EVs enriched in pro-angiogenic factors such as VEGF, VEGFR2, monocyte chemoattractant protein-1 (MCP-1), angiogenin, IL-6, Tie-2/TEK and insulin-like growth factor (IGF). It therefore seems that a hypoxic microenvironment may also play a role in the enrichment of EVs with proangiogenic factors as the same was observed by Salomon et al.[56] for EVs isolated from placenta-derived MSCs. Furthermore, a recent study by Anderson et al. [57] who carried out a comprehensive proteomic analysis of MSC EVs identified a key role played by the NFkB signalling pathway in EV mediated angiogenesis.

5. Conclusions

Angiogenesis is a well-regulated process that plays a crucial role in embryo development, vascular homeostasis in mature organisms, and during tissue regeneration. An altered process of angiogenesis has been implicated in various pathological conditions including tumours and inflammation. Besides soluble factors, EVs have recently emerged as mediators of autocrine/paracrine activity in new vessel formation.

Angiogenic EVs are released from different cell types. Their biological activity, depending on the cell of origin, the content they carry, and the situation in which they are released in, may have either beneficial or detrimental effects. The microenvironment, in particular hypoxia, may modulate the release as well as the quality of their cargo. Although EVs released by normal endothelial cellsmay have a role in endothelial homeostasis, those derived from tumour or inflammatory cells could promote an enhanced angiogenic response pathological in nature. On the other hand, EVs released from stem/progenitor cells have been shown to play a more therapeutic role by limiting ischemic injuryand

contributing towards tissue regeneration. Therefore, there have been suggestions to apply stem/progenitor cell derived EVs as a potential tool in therapy. Nevertheless, it should be acknowledged that native EVs are heterogeneous in nature and in content which, albeit may be advantageous as they may deliver multiple molecules that could have a synergistic effect on angiogenesis. However, this heterogeneity may present a problem during the standardization process of EV preparations for eventual therapeutic application. It is therefore important to identify and categorize the active molecules carried by these EVs, as this may allow the generation of engineered EVs enriched with potential angiogenic molecules as well as specific surface molecules that would facilitate endothelial targeting.

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References

[1] P. Carmeliet, R.K. Jain, Molecular mechanisms and clinical applications of angiogenesis, Nature 473 (2011) 298–307, http://dx.doi.org/10.1038/nature10144.

[2] G. Raposo, W. Stoorvogel, Extracellular vesicles: exosomes, microvesicles, and friends, J. Cell Biol. 200 (2013) 373–383.

[3] M.A. Lopez-Verrilli, F.A. Court, Exosomes: mediators of communication in eukaryotes, Biol. Res. 46 (2013) 5–11.

[4] F. Lavialle, S. Deshayes, F. Gonnet, E. Larquet, S.G. Kruglik, N. Boisset, et al., Nanovesicles released by Dictyostelium cells: a potential carrier for drug delivery, Int. J. Pharm. 380 (2009) 206–215.

[5] M.L. Rodrigues, J.D. Nosanchuk, A. Schrank, M.H. Vainstein, A. Casadevall, L. Nimrichter. Vesicular transport systems in fungi. Future Microbiol 6(2011):1371–1381.

[6] M. Regente, M. Pinedo, M. Elizalde, L. De La Canal, Apoplastic exosome-like vesicles: a new way of protein secretion in plants? Plant Signal. Behav. 7 (2012) 544–546.

[7] S. Liegeois, A. Benedetto, J.M. Garnier, Y. Schwab, M. Labouesse, The V0-ATPase mediates apical secretion of exosomes containing Hedgehog-related proteins in Caenorhabditis elegans, J. Cell Biol. 173 (2006) 949–961.

[8] C. Korkut, B. Ataman, P. Ramachandran, J. Ashley, R. Barria, N. Gherbesi, et al., Transsynaptic transmission of vesicular Wnt signals through Evi/Wntless, Cell 139 (2009) 393–404.

[9] M. Record, C. Subra, S. Silvente-Poirot, M. Poirot, Exosomes as intercellular signalosomes and pharmacological effectors, Biochem. Pharmacol. 81 (2011) 1171–1182.

[10] C. Gai, A. Carpanetto, M.C. Deregibus, G. Camussi, Extracellular vesicle-mediated modulation of angiogenesis, Histol. Histopathol. 11 (2015) 11708.

[11] S.J. Gould, G. Raposo, As we wait: coping with an imperfect nomenclature for extracellular vesicles, J. Extracell. Vesicles 2 (2013), http://dx.doi.org/10.3402/jev.v2i0. 20389 (eCollection 2013).

[12] M. Colombo, G. Raposo, C. Théry, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289, http://dx.doi.org/10.1146/annurev-cellbio-101512-122326.

[13] M. Colombo, C. Moita, G. van Niel, J. Kowal, J. Vigneron, P. Benaroch, et al., Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles, J. Cell Sci. 126 (2013) 5553–5565.

[14] C. Bissig, J. Gruenberg, ALIX and the multivesicular endosome: ALIX in Wonderland, Trends Cell Biol. 24 (2014) 19–25.

[15] K. Trajkovic, C. Hsu, S. Chiantia, L. Rajendran, D. Wenzel, F. Wieland, et al., Ceramide triggers budding of exosome vesicles into multivesicular endosomes, Science 319 (2008) 1244–1247.

[16] Y. Fang, N. Wu, X. Gan, W. Yan, J.C. Morrell, S.J. Gould, Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes, PLoS Biol. 5 (2007), e158.

[17] M. Yáñez-Mó, P.R. Siljander, Z. Andreu, et al., Biological properties of extracellular vesicles and their physiological functions, J. Extracell. Vesicles 4 (2015) 27066, http://dx.doi.org/10.3402/jev.v4.27066 (eCollection 2015).

[18] V. Cantaluppi, L. Biancone, F. Figliolini, S. Beltramo, D. Medica, M.C. Deregibus, et al., Microvesicles derived from endothelial progenitor cells enhance neoangiogenesis of human pancreatic islets, Cell Transplant. 21 (2012) 1305–1320.

[19] S. Bruno, C. Grange, M.C. Deregibus, R.A. Calogero, S. Saviozzi, F. Collino, et al., Mesenchymal stem cell-derived microvesicles protect against acute tubular injury, J. Am. Soc. Nephrol. 20 (2009) 1053–1067.

[20] A. Vargas, S. Zhou, M. Éthier-Chiasson, D. Flipo, J. Lafond, C. Gilbert, et al., Syncytin proteins incorporated in placenta exosomes are important for cell uptake and show variation in abundance in serum exosomes from patients with preeclampsia, FASEB J. 28 (2014) 3703–3719.

[21] I. Parolini, C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. De Milito, et al., Microenvironmental pH is a key factor for exosome traffic in tumor cells, J. Biol. Chem. 284 (2009) 34211–34222.

[22] A. Montecalvo, A.T. Larregina, W.J. Shufesky, D.B. Stolz, M.L. Sullivan, J.M. Karlsson, et al., Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes, Blood 119 (2012) 756–766.

[23] E. Cocucci, G. Racchetti, J. Meldolesi, Shedding microvesicles: artefacts no more, Trends Cell Biol. 19 (2009) 43–51.

[24] T. Tian, Y.L. Zhu, Y.Y. Zhou, G.F. Liang, Y.Y. Wang, F.H. Hu, et al., Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR21 delivery, J. Biol. Chem. 289 (2014) 22258–22267

[25] A. Sarkar, S. Mitra, S. Mehta, R. Raices, M.D. Wewers, Monocyte derived microvesicles deliver a cell death message via encapsulated caspase-1, PLoS One 4 (2009), e7140.

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

[27] K. Al-Nedawi, B. Meehan, J. Micallef, V. Lhotak, L. May, A. Guha, et al., Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells, Nat. Cell Biol. 10 (2008) 619–624, http://dx.doi.org/10.1038/ncb1725.

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

[29] M.C. Deregibus, V. Cantaluppi, R. Calogero, M. Lo Iacono, C. Tetta, L. Biancone, et al., Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA, Blood 110 (2007) 2440–2448.

[30] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J.J. Lee, J.O. Lötvall, Exosomemediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659.

[31] G. Raposo, H.W. Nijman, W. Stoorvogel, R. Liejendekker, C.V. Harding, C.J. Melief, et al., B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (1996) 1161–1172.

[32] C.W. Kim, H.M. Lee, T.H. Lee, C. Kang, H.K. Kleinman, Y.S. Gho, Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin, Cancer Res. 62 (2002) 6312–6317.

[33] G. Taraboletti, S. D'Ascenzo, P. Borsotti, R. Giavazzi, A. Pavan, V. Dolo, Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicleassociated components by endothelial cells, Am. J. Pathol. 160 (2002) 673–680.

[34] D. Millimaggi, M. Mari, S. D'Ascenzo, E. Carosa, E.A. Jannini, S. Zucker, et al., Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells, Neoplasia 9 (2007) 349–357.

[35] Z. Andreu, M. Yáñez-Mó, Tetraspanins in extracellular vesicle formation and function, Front. Immunol. 5 (2014) 442.

[36] I. Nazarenko, S. Rana, A. Baumann, J. McAlear, A. Hellwig, M. Trendelenburg, et al., Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosomeinduced endothelial cell activation, Cancer Res. 70 (2010) 1668–1678, http://dx. doi.org/10.1158/0008-5472.CAN-09-2470.

[37] S. Gesierich, I. Berezovskiy, E. Ryschich, M. Zöller, Systemic induction of the angiogenesis switch by the tetraspanin D6.1A/CO-029, Cancer Res. 66 (2006) 7083–7094.

[38] J. Skog, T. Würdinger, S. van Rijn, D.H. Meijer, L. Gainche, M. Sena-Esteves, et al., Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. 10 (2008) 1470–1476, http://dx.doi.org/10.1038/ncb1800.

[39] J.E. Park, H.S. Tan, A. Datta, R.C. Lai, H. Zhang, W. Meng, et al., Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes, Mol. Cell. Proteomics 9 (2010) 1085–1099, http://dx.doi.org/10.1074/mcp.M900381-MCP200.

[40] H.W. King, M.Z. Michael, J.M. Gleadle, Hypoxic enhancement of exosome release by breast cancer cells, BMC Cancer 12 (2012) 421, http://dx.doi.org/10.1186/1471-2407-12-421.

[41] V. Cantaluppi, S. Gatti, D. Medica, F. Figliolini, S. Bruno, M.C. Deregibus, et al., Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia–reperfusion injury by microRNA-dependent reprogramming of resident renal cells, Kidney Int. 82 (2012) 412–427, http://dx.doi.org/10.1038/ki.2012.105.

[42] T. Asahara, T. Murohara, A. Sullivan, M. Silver, R. van der Zee, T. Li, et al., Isolation of putative progenitor endothelial cells for angiogenesis, Science 275 (1997) 964–967.

[43] M.C. Yoder, Human endothelial progenitor cells, Cold Spring Harb. Perspect. Med. 2 (2012) a006692, http://dx.doi.org/10.1101/cshperspect.a006692.

[44] H. Sheldon, E. Heikamp, H. Turley, R. Dragovic, P. Thomas, C.E. Oon, et al., New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes, Blood 116 (2010) 2385–2394.

[45] A. Ranghino, V. Cantaluppi, C. Grange, L. Vitillo, F. Fop, L. Biancone, et al., Endothelial progenitor cell-derived microvesicles improve neovascularization in a murine model of hindlimb ischemia, Int. J. Immunopathol. Pharmacol. 25 (2012) 75–85.

[46] A. Eirin, S.M. Riester, X.Y. Zhu, H. Tang, J.M. Evans, D. O'Brien, et al., MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells, Gene 551 (2014) 55–64.

[47] T. Lopatina, S. Bruno, C. Tetta, N. Kalinina, M. Porta, G. Camussi, Platelet-derived growth factor regulates the secretion of extracellular vesicles by adipose mesenchymal stem cells and enhances their angiogenic potential, Cell Commun. Signal. 12 (2014) 26.

[48] T. Lopatina, A. Mazzeo, S. Bruno, C. Tetta, N. Kalinina, R. Romagnoli, et al., The angiogenic potential of adipose mesenchymal stem cell-derived extracellular vesicles is modulated by basic fibroblast growth factor, J. Stem Cell Res. Ther. 4 (2014) 245, http://dx.doi.org/10.4172/2157-7633.1000245.

[49] G. Togliatto, P. Dentelli, M. Gili, S. Gallo, M.C. Deregibus, E. Biglieri, et al., Obesity reduces the pro-angiogenic potential of adipose tissue stem cell derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications, Int. J. Obes. 40 (2016) 102–111.

[50] M.F. Pittenger, B.J. Martin, Mesenchymal stem cells and their potential as cardiac therapeutics, Circ. Res. 95 (2004) 9–20.

[51] R.C. Lai, F. Arslan, M.M. Lee, N.S. Sze, A. Choo, T.S. Chen, et al., Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury, Stem Cell Res. 4 (2010) 214–222.

[52] F. Arslan, R.C. Lai, M.B. Smeets, L. Akeroyd, A. Choo, E.N. Aguor, et al., Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodelling after myocardial ischemia/reperfusion injury, Stem Cell Res. 10 (2013) 301–312.

[53] S. Bian, L. Zhang, L. Duan, X. Wang, Y. Min, H. Yu, Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model, J. Mol. Med. (Berl) 92 (2014) 387–397.

[54] B. Zhang, X. Wu, X. Zhang, Y. Sun, Y. Yan, H. Shi, et al., Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/β-catenin pathway, Stem Cells Transl. Med. 4 (2015) 513–522.

[55] J. Chen, Z. Liu, M.M. Hong, H. Zhang, C. Chen, M. Xiao, et al., Proangiogenic compositions of microvesicles derived from human umbilical cord mesenchymal stem cells, PLoS One 9 (2014), e115316, http://dx.doi.org/10.1371/journal.pone.0115316 (eCollection 2014).

[56] C. Salomon, J. Ryan, L. Sobrevia, M. Kobayashi, K. Ashman, M. Mitchell, et al., Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis, PLoS One 8 (2013), e68451, http://dx.doi.org/10. 1371/journal.pone.0068451.

[57] J.D. Anderson, H.J. Johansson, C.S. Graham, M. Vesterlund, M.T. Pham, C.S. Bramlett, et al., Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via NFkB signaling, Stem Cells (2015), http://dx. doi.org/10.1002/stem.2298.