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Review

Extracellular vesicles as new players in angiogenesis

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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.

Contents

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.

Abbreviations: EVs, extracellular vesicles; MVBs, multivesicular bodies; ESCRT, exosomal sorting complex required for transport; DCs, dendritic cells; lncRNA, long non-coding 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 factor-receptor; IL-3, interleukin-3; IL-6, interleukin-6; IL-8, interleukin-8; TIMP-1 and -2, tissue inhibitors of metalloproteinases-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.

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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 [3–7] 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 [11–13]. 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 [12–15]. 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) [28–30] 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 proteins necessary 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 MAPK and 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].