Tumor endothelial cell-derived extracellular vesicles contribute to tumor microenvironment remodeling

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Abstract
Cancer progression involves several biological steps where angiogenesis is a key tumorigenic phenomenon. Extracellular vesicles (EVs) derived from tumor cells and other cells in the tumor microenvironment (TME) help modulate and maintain favorable microenvironments for tumors. Endothelial cells (ECs) activated by cancer-derived EVs have important roles in tumor angiogenesis. Interestingly, EVs from ECs activate tumor cells, i.e. extracellular matrix (ECM) remodeling and provide more supplements for tumor cells. Thus, EV communications between cancer cells and ECs may be effective therapeutic targets for controlling cancer progression. In this review, we describe the current knowledge on EVs derived from ECs and we examine how these EVs affect TME remodeling.

Keywords: Extracellular vesicles, Tumor microenvironment, Angiogenesis, Remodeling, Targeted therapy

Background
Cancer is a complex multifactorial disease where normal cells acquire multiple traits to facilitate prolonged cell survival [1]. Tumor occurrence and development is inseparable from the tumor microenvironment (TME) which is a highly heterogeneous ecosystem incorporating: 1) immunocytes (T/B lymphocytes, tumor-associated macrophages, dendritic cells (DCs), natural killer cells (NKs), myeloid-derived suppressor cells, and neutrophils); 2) stromal cells (cancer-associated fibroblasts (CAF), pericytes, and mesenchymal stromal cells); 3) extracellular matrix (ECM) and other secreted molecules (extracellular vesicles (EVs), growth factors, cytokines, and chemokines); and 4) blood and lymph vessel networks [2]. Such cell-to-cell interactions can lead to TME remodeling and may exert a significant impact on cancer progression and metastasis, drug resistance, and immunosuppression.

Recently EVs, also known as exosomes (Exs), have been recognized as crucial signaling mediators in TME regulation. EVs are double-membrane vesicle-like bodies which are shed from cell membranes or secreted by cells, with diameters ranging from 40 to 1000 nm. EVs are mainly composed of microvesicles (MVs) and Exs [3]. To explore this topic more comprehensively, the terms “EVs” and “Exs” are used interchangeably. EV-mediated communication networks between tumor and non-tumor cells appears to be involved from tumor growth to metastasis. For example, a gastric cancer study reported that the EV cargoes inhibin subunit βA and thrombospondin 2 were secreted by CAF-derived EVs to the TME to promote cancer [4]. Glioblastoma-derived EVs dramatically promoted neural progenitor cell proliferation and migration via the PI3K/Akt pathway [5]. A recent study also reported that EV transmitted long-non coding (lnc)ARSR which promoted sunitinib resistance in renal cancer [6]. EVs released by tumor cells could also remodel the microenvironment by activating CAF [7] and promoting...
immune escape [8] and angiogenesis [9]. Among the multiple stromal cell types in the TME, endothelial cells (ECs) are an enriched source of circulating EVs as they transfer information to adjacent cell types [10].

Several studies have now demonstrated that stromal cell-derived EVs promote tumorigenic phenotypes and that tumor-derived EVs modify the host stroma. However, the effects of EVs released from ECs on TEM are rarely studied. In this review, the role of EC-derived EVs is highlighted to examine their contribution to tumor cells and immune cells.

**Loading and releasing EVs**

Previous studies have confirmed that endosome sorting complexes required for transport (ESCRT) are widely accepted regulatory mechanisms for EV processing, formation, and release. ESCRTs are composed of ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III, and vacuolar protein sorting-associated protein 4 (VPS4) [11]. ESCRT-0 initiates pre-ubiquitinated protein sorting and forms intraluminal vesicles (ILVs). Under the action of ESCRT-I/II, ILVs undergo membrane fusion to form multi-vesicular bodies (MVBs). Inside the cell, MVB parts are degraded by lysosomes, while the remaining components move toward cell membranes and fuse with them. At this time, MVBs are still connected to the membrane surface and ESCRT-III forms a spiral structure during this process. This structure shrinks the MVB neck and the cell membrane. At the same time, the VPS4 ATPase directly or indirectly hydrolyzes the MVB through hydrolysis, after which MVBs are released extracellularly. EVs are also formed via an ESCRT-independent mechanism; Pmel17 regulates ILV production and affects EV formation via its luminal domain [12]. Also, the four-transmembrane CD63 protein mediates melanosome invagination in an ESCRT-independent manner [13]. The PLP protein is transferred from lipid-rich endosomal membranes to ILVs via an ESCRT-independent manner [14]. In addition to ESCRT-mediated pathways, Wei et al. identified phosphorylates RAB31 drives EGER entry into multivesicular endosomes (MVE) to form ILVs and Exs, which is dependent of flotillin proteins in lipid raft microdomains instead of ESCRT [15].

EV-release from inside the cell to the outside is synergistically coordinated via several steps which mainly act on MVB and cell membrane separation processes. Studies have confirmed the most important factors mediating EV release are GTPases, including Rab and RAL GTPases. Up to now, nine GTPases have been implicated in EV release, including Rab2B, Rab5, Rab7, Rab9A, Rab11, Rab27A, Rab27B, Rab35, and RAL [16]. Rabs have vital regulatory roles transporting MVBs to subcellular locations and fusing with cell membranes [17]. MVBs can be directly or indirectly bound to actin and microtubule scaffolds to facilitate intracellular targeted transport. Interestingly, Rab11 and its family assist MVB transport via actin and dynein [18]. The process from MVBs to EVs requires not only motor protein dynamics, but also MVB separation from cell membranes which is particularly important. Although the specific mechanisms whereby Rab participates in MVB dissociation at the cell membrane have not been confirmed, it is hypothesized Rab initiates the direct or indirect assembly of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes (Fig. 1).

The SNARE complex has vital roles in MVB fusion with cell membranes, especially when promoting MVB release. The SNARE complex consists of two components, v- and t-SNAREs. The v-SNARE is located on the vesicle while the t-SNARE is located on the presynaptic membrane. Both v- and t-SNAREs pair up and form a complex [19]; during this formation, the released energy draws MVBs closer to presynaptic membranes and promotes MVB membrane fusion. A recent study in human leukemic K562 cells confirmed that VAMP7 (a v-SNARE protein) was involved in MVB plasma membrane fusion and exosomal release [20]. In mammals, SYX-5 (a t-SNARE proteins) was also involved in MVB fusion and promoted EV release [21]. In tumor cells, the key glycolysis enzyme PKM2 stabilized SNAREs via SNAP-23 (a t-SNARE protein) phosphorylation to promote external EV release [22]. These studies confirmed that mediating EV release was a complex process involving multiple steps and factors. While the regulatory mechanism is not the same in different cells, the main processes often involve GTIPases and SNARE complexes. These observations raise an
important question in EV research: do different sorting mechanisms determine the loading of specific molecules into EVs? By interfering with this sorting mechanism, it is possible to influence content loading in EVs to alter intercellular substance exchange in the TME.

**EV uptake**

EVs released into the extracellular space are not only re-absorbed and used by EV-derived origin cells, but are also taken up by other cells in the microenvironment. Cells take up EVs mainly via cell membrane fusion, endocytosis, and binding with specific surface receptors. Additionally, when carrying functional molecules, EVs transfer information between cells and mediate many physiological and pathological processes. Tian et al. reported that rat pheochromocytoma PC12 cell-derived Exs entered and delivered microRNAs into bone marrow-derived mesenchymal stromal cells and down-regulated the expression of transforming growth factor β receptor II and tropomyosin-1 [23]. In non-neoplastic diseases, ECs transduced with HDAdXMozAntimiR33a5p released Exs that transferred anti-miR-33a-5p to other intimal cell types, thereby upregulating cholesterol efflux from these cells. [24]. At present, the decisive factors determining EV uptake remain unclear. Edgar et al. reported that the protein tether in mediating the attachment to the cell surface and signal transmission of EVs [25]. Christianson et al. confirmed that on the surface of receptor cells, the heparin sulfate proteoglycan (HSPG) acted as an EV receptor rather than just an attachment site on the EV surface [26]. In mammals, differences in integrin family expression often affect EV uptake by receptor cells [27]. Importantly, some mechanisms can inhibit uptake; CD47 on EV surfaces protects EVs from engulfment by recipient cells and improves EV stability in the microenvironment [28].

Tumor-derived EVs taken up by ECs often facilitate angiogenesis signaling and stimulate blood vessel formation [29]. In ECs, EV take-up is interceded via the collaboration of EV surface proteins, e.g., tetraspanins with the membrane receptors of beneficiary cells [30]. In the TME, cell surface Tspan8-CD49d complexes from EVs derived from tumors are incorporated by vascular rodent ECs to improve EC proliferation, migration, and activation [31]. EVs bearing Tspan8-4 complexes bind with intercellular adhesion molecule-1 (ICAM-1) and then were compassed by rat ECs [32]. In the absence of content delivery, EVs send messages to beneficial cells via surface contacts, e.g., EVs containing major histocompatibility complex (MHC)-peptide complexes can activate T cells via surface receptors [33]. Within 24 h, ECs internalize EVs produced by cancer cells using an internalization pathway. This was previously confirmed in studies where ECs easily captured PKH26-dyed EVs during the first 4 h [34]. After internalization, EVs were immediately directed to the perinuclear zone; they moved to the cell periphery and entered advanced pseudopods when tubules were formed in vitro. After complete remodeling, adjacent ECs transported EVs to other ECs and cells in the TME via nanoparticle structures [35].

**EC-derived EV characterization, separation, and roles in tumor progression**

EVs derived from ECs are implicated in several physiological and pathological conditions [36–38]. The particular secretome of endothelia mirrors their molecular diversity and possibly supports EC plasticity and adaptation within or between different vascular beds while also significantly impacting circulating cells, including immune cells in blood or lymph [39]. The markers CD63 and CD81 are specifically expressed in EVs. In addition, several other EV-specific markers, i.e. CD31, CD54, CD62E, CD105, CD144, CD146, and von Willebrand factor also occur on EVs [32, 33]. Moreover, EC-derived EVs also deliver proteins such as ICAMs, vascular endothelial (VE)-cadherin, E-selectin, platelet EC adhesion molecule-1, endoglin, and endothelial nitric oxide synthase [40–42]. While the exosomal endothelial markers CD54 and CD62E are upregulated by various stimuli, CD31 and CD105 are specifically increased by apoptosis [43]. CD62E and CD144 are exclusively expressed by ECs, while the aforementioned molecules are not (Fig. 2). To improve specificity and sensitivity, combined multicolor antibodies (CD31+/CD41−, CD31+/CD42b−, and CD105+/CD45−) and monochrome composite markers (CD144+ CD105− and CD146+ CD105−) are used to isolate EC-derived EVs. Because ECs express CD62E and CD144 [43], EC-delivered EVs can be specifically separated, identified, and quantified from plasma and various tissues by immunoaffinity (CD144/CD62E antibodies) or nonscale fluorescence-activated cell sorting, which is a highly promising and rapidly advanced method for EV separation and characterization [44].

EV cargoes are closely related to donor cells, and the substances in EVs released by different cells often have significant differences [45]. Current research has confirmed that EVs released by ECs contain high levels of IncRNAs and protein components. These contents exert different functions during cancer; some inhibit cancer progression, including miR-503 [46] and miR-126 [47], while others promote cancer progression, including MALAT1 [48], S100A16 [49], delta-like protein 4 [50], angiopoietin-2 (Ang2) [51], and carciinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1) [52]. For non-neoplastic diseases, EV contents also exert important regulatory roles as they mediate EC function,
with miR-125a [53], miR-210 [54], miR-375 [55], miR-214 [56], lysyl oxidase like-2 (LOXL2) [57], and HSP70 [58] having significant roles in this process. In heart disease, miR-10b-5p [59], miR-146a [60], and miR-19a [61] are important molecules, while for vascular smooth muscle cell regulation, miR-143 [62] and versican [63] are highly significant. In atherosclerosis, miR-505 [64] and miR-155 [65] also have similarly important functions. In chronic obstructive pulmonary disease, R-191 predicts the potential function of Exs as paracrine effectors [53]. TGF-β1 could ameliorate renal structure and function [66]. Cytomegalovirus could stimulate allogeneic CD4⁺ memory T cells [67]. The effects of EV release on tumor cells is mainly facilitated via these cargoes. e.g., miR-503 in EVs regulate the proliferation and invasion of triple-negative breast cancer cells [46]. Also, stressed human umbilical vein endothelial cells (HUVECs) release EVs containing miR-126, which significantly inhibits tumor cell growth [47]. In addition to releasing “tumor-suppressing” EVs, ECs also release “tumor-promoting” EVs. The EVs released from human brain microvascular ECs promote S100A16 expression in lung cancer cells, increase lung cancer cell and anti-apoptotic activity, thereby promoting lung cancer development. ECs also release EVs carrying Ang2 which promotes tumor progression [51]. Under stress conditions, CEACAM1 in EVs inhibits T cell activation, which may be an important factor promoting tumor progression. Our previous studies showed that YAP1 inhibition in HUVECs was associated with EV release and increased hepatocyte carcinoma (HCC) invasion and metastasis (Fig. 3). Our results suggested that during tumor treatment, EVs were a possible reason for treatment failure [48] (Table 1).

The role of EVs toward immune cells

EVs trigger and regulate immune responses, especially in cancer [68]. Several studies have reported that various molecules (adhesions, heat shock proteins, molecules involved in membrane trafficking and ESCRTs), immune response molecules (e.g., MHC class I and II proteins), immune receptors, ligands (FasL, TRAIL, PD-L1, NKG2D ligands), and co-stimulatory molecules are distinct cargoes in some specialized EVs. Many immunocytes, such as DCs [69], NKs, and T cells [70] are activated by ECs harboring non-classical or HLA-E, MICA, and other NKG2D ligands. In cardiovascular disease, including vascular inflammation and atherosclerotic plaque formation, EVs mediate cross-talk between recipient cells and ECs, and reprogram them toward a pro- or anti-inflammatory stance. The pro-inflammatory molecules with chemotactic mediators, including ICAM-1, CCL-2, IL-6, IL-8, CXCL-10, CCL-5, and TNF-α were released by EVs and taken up by monocytes and HUVECs [71]. In anti-inflammatory aspect displays, ECs released EVs containing miRNA-222 to reduce endothelial ICAM-1 expression both in vitro and in vivo [72]. In addition, miR-10a was transferred to monocytes from EC-EVs and could repress inflammatory signaling through the targeting of several components of the NF-κB pathway, including IRAK4 [73].
Lipopolysaccharide induces the neutrophil secretion of EVs containing miR-122-5p which functions in oxidative stress, apoptosis, and increased brain microvascular EC permeability [74]. EVs released by apoptotic ECs are reported as having unique transcriptomic characteristics and contain non-coding RNA (ncRNA) sequences (mitochondrial transfer RNA, U1 small nuclear RNA, and pathogen-like endogenous retro-elements) with immunostimulatory potential. These EVs from apoptotic ECs may be recognized by RIGI-like receptors and toll like receptors (TLRs) (TLR3, TLR7, and TLR8) and possibly initiate innate immune responses [75].

The circuitous connection between certain EVs and tumors warrants further investigation. Specific ncRNAs have been identified as cargoes in specialized EVs released by ECs; interestingly, the same ncRNAs regulate tumor progression in other studies. We have no proof of a relationship between EC-EV-ncRNA-tumor, yet this gives us a sensible estimate concerning the connection among these elements. Zhao et al. found that miR-503 downregulated immune function in esophagus carcinoma [76], while Bovy et al. confirmed miR-503 was contained in EC EVs during breast cancer neoadjuvant chemotherapy [46]. LncRNAs also have important regulatory roles in tumor immunity. We previously demonstrated that ECs release EVs which carried MALAT1 [48], while Hou et al. reported that MALAT1 promoted immunosuppressive properties in HCC cells [77]. Primary mouse lung ECs may release EVs containing Ang2, while coincidentally, Schmittnaegel et al. verified that ANG-2 inhibition elicited antitumor immunity which was enhanced by PD-1 checkpoint blockade [78]. In addition to ncRNAs, proteins from EVs released by ECs also have important functions in tumor immunity. S100A6 overexpression possibly impaired the infiltration and cytolytic activity of CD8⁺ T cells via the focal adhesion-Ras-stimulating signaling pathway in pancreatic cancer [79]. Brain microvascular EC-EVs contained S100A16...
which is an immune-related prognostic biomarker and therapeutic target for low-grade glioma [80]. These results suggest EVs released from ECs may be important factors regulating tumor immunity. Anti-tumor immuno-therapy targeting EC-EVs may an important anti-tumor therapy mechanism in the future.

EC derived EV based therapeutic strategies

EVs containing various surface adhesion proteins have several advantages as delivery vectors for cancer gene therapy. As cancers are often associated with aberrant EV formation and release, the inhibition of this pathological process could be an emerging approach for cancer treatment.

EVs as novel biocarriers for drug delivery

EC-derived EVs are advantageous for drug targeted delivery as they carry biological substances to recipient cells. Using in vitro and ex vivo techniques, including freeze-thaw cycles, incubation, saponin infiltration, sonication, and extrusion procedures, several therapeutic drugs have been loaded into EVs [81]. Thus, EVs are stable, and drug structure and activity remains unchanged after EV loading, even when stored at freezing temperatures. Recent studies suggested that EV delivery and effective cellular uptake could be improved by altering the EV surface with an Arg-Gly-Asp-D-Tyr-Lys peptide [82] or promoting efficient cytosolic release using enhancing cationic lipids [83]. Unfortunately, few studies have reported EC-derived EVs for delivering drugs in experimental or clinical studies, while other cell studies reported that EVs carrying anticancer drugs are promising new therapeutic strategies in animal experiments as a promising approach for cancers [84]. In addition to undergoing a phase I clinical trial (NCT01294072), these therapeutic strategies are being used for EC/EV drug delivery for cardiovascular diseases.

| Table 1 | The role of EC-derived EVs in tumor and non-tumor diseases |
| --- | --- | --- |
| **EVs on tumor cells** | Parent cell | Biological function | Reference |
| NcRNA miR-503 HUVECs | Inhibits cancer proliferation and invasion | [46] |
| miR-126 HUVECs | Inhibits the growth of tumor cells | [47] |
| MALAT1 HUVEC | Promotes invasion in HCC | [48] |
| Protein S100A16 HBMVECs | Promotes SCLC survival in brain | [49] |
| Dll4 HMVEC | Promotes angiogenesis | [50] |
| Ang2 Primary mouse lung endothelial cells | Promotes tumor growth | [51] |
| CEACAM1 human endothelial cell line AS-M.5 | Modulate immune response, tumor progression, metastasis and angiogenesis | [52] |

| **EVs on non-neoplastic diseases** | Parent cell | Biological function | Reference |
| NcRNA miR-10b-5p HUVEC | Reduced inflammation in cardiovascular disease | [59] |
| miR-125a Human lung microvascular | Modulate the phenotype of distant endothelial cells of large systemic | [53] |
| miR-143 HUVEC | Control smooth muscle cell phenotype | [62] |
| miR-146a HUVEC | Decrease in metabolic activity in peripartum cardiomyopathy | [60] |
| miR-191 Human lung microvascular | Predicting potential function of exosomes as paracrine effectors in CIPD | [53] |
| miR-210 Endothelial progenitor cells | Protective effects on ECs against H/R injury | [54] |
| miR-375 Endothelial progenitor cell | Rescued the cell protection activity | [55] |
| miR-214 HMEC-1 | Blood vessel formation | [56] |
| miR-505 HUVEC | Inducing Oxidative stress and inflammation in atherosclerosis | [64] |
| miR-155 HUVEC | Modulate the macrophage phenotype in atherosclerosis | [65] |
| miR-19a HUVEC | Improved vascularization and cardiac function, decreased myocardial fibrosis | [61] |
| Protein TGF-β1 Glomerular endothelial cells | Ameliorate renal structure and function | [66] |
| Versican HUVEC | Regulate vascular smooth muscle cells calcification/senescence in high glucose condition | [63] |
| LOXL2 Human microvascular endothelial cells | Altered abundances after exposure of their producing cells to cellular stress | [57] |
| Cytomegalovirus HUVEC | Stimulate allogeneic CD4+ Memory T Cells | [67] |
| HSP70 Rat aortic endothelial cells | Induction of monocyte activation and endothelial cell adhesion | [58] |
EVs carry small interfering RNAs (siRNAs)

EVs carry RNAs, which facilitate communications between cells. Critically, siRNA delivery to ECs and other cells could be an important gene therapy strategy. Because protein expression is suppressed by siRNAs via specific posttranscriptional sequences, siRNAs offer a unique opportunity to treat multiple ECs related to cancer. Recently, across different fields, several siRNA vectors have been used, including viruses, synthetic polymers, and lipid-based carriers. When compared with other RNA vectors, EVs when used as nanoparticles to transfer siRNAs, are more advantageous in retaining bioavailability, delivery efficiency, and compatibility [85]. Moreover, siRNA-containing EVs cross the endothelial barrier and guide siRNAs to target specific tissues and cells [85]. Also, as these molecules easily pass the blood–brain barrier, EVs are extensively used as small RNA carriers to treat brain tumors [86]. A recent study showed that EVs containing siRNA knocked down target gene expression by 50%–90% in different cancer cells [87]. However, few investigations have provided evidence of specific ECs releasing EVs to deliver siRNA. Yang et al. demonstrated that siRNA-VEGF delivery in EC-EVs suppressed VEGF transcription and translation in co-cultured GBM-astrocytoma cells, and showed that siRNA delivery silenced target genes and could be used as a potential therapeutic strategy for cancer [88].

Reducing the abnormal release of EVs

In terms of abnormal EV-release related to cancer, several studies emphasized the biological ways of EVs to verify the key way to suppress the exocrine of EVs. nSMase took part in lipid raft and worked as a ubiquitous enzyme to improve EV formation [89]. These results suggested EV formation and release were blocked by siRNAs against nSMase2. Menck et al. reported that nSMase-2 inhibition by GW4869 or RNA interference decreased Exs secretion but increased MV secretion from plasma membranes both in vitro and in vivo [90]. Furthermore, GW4869 prevented abnormal EV release and improved abnormal vascular remodeling [91]. Rab proteins are key regulators of EV secretion, e.g., Rab27 participates in EV exocytosis. Also, EV release is reduced by RAB27 inhibition [92]. In colorectal cancer cells, RAB27 knockdown inhibited EV release and the exosomal-related proliferation and migration of ECs [93].

Conclusions and perspectives

Angiogenesis is an essential step in tumorigenesis and development. Tumor cell metastasis to distant organs and the rapid proliferation of focal tumor cells are inseparable from new blood vessel formation. EVs are important mediators during material exchange processes between tumor cells and ECs. EVs released by tumor cells carry proteins and ncRNAs to promote new tumor blood vessel formation. ECs in the TME also release EVs to promote tumor progression. Currently, the function and molecular mechanisms underpinning the EC release of EVs are unclear and require further study. However, EV sources, their loading, and related processes are affected by their cell origin, changes in the TME, and target cells. Therefore, targeting these EV characteristics could provide an intervention for regulating tumor angiogenesis. In particular, EVs are produced in the body and effectively avoid immune surveillance, therefore, they have a great potential as a targeted therapy for drug delivery in clinical settings. Based on these observations, cross-talk between ECs and other cells can be mediated by EVs and contribute to TME remodeling. EC-derived EVs are expected to become important targets for tumor treatments in the future.

Abbreviations

EVs: Extracellular vesicles; TME: Tumor environment; TAM: Tumor-associated macrophages; DC: Dendritic cells; NK: Natural killer cells; MDSC: Myeloid-derived suppressor cells; CAFs: Cancer-associated fibroblasts; ECM: Extracellular matrix; MVs: Microvesicles; Exs: Exosomes; MVE: Multivesicular endosomes; ECs: Endothelial cells; HCC: Hepatocyte carcinoma; ESCRT: Endosome sorting complexes required for transport; MV8: Multi-vesicular body; VPS4: Vacuolar protein sorting-associated protein 4; ILV: Intraluminal vesicles; MV8: Multivesicular bodies; SNARE: Soluble N-ethylmaleimide-sensitive protein attachment receptor; ANGPT1: Angiopoietin-1; BMSCs: Bone marrow-derived mesenchymal stromal cells; TGFβRII: Transforming growth factor β receptor II; TPM1: Tropomyosin-1; HSPG: Heparin sulfate proteoglycan; CA9: Carbonic anhydrase 9; miRNA: MicroRNA; IncRNA: Long-noncoding RNA; WVF: Von Willebrand factor; ICAM: Intercellular cell adhesion molecule; MHC: Major histocompatibility complex; PECAM-1: Platelet EC adhesion molecule-1; eNOS: Endothelial nitric oxide synthase; nanoFACS: Nanoscale fluorescence activated cell sorting; DII4: Delta-like protein 4; LOXL2: Lysyl Oxidase Like-2; Ang2: Angiopoietin-2; CEACAM1: Carcinoembryonic antigen-related cell adhesion molecule-1.

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Declarations

Competing interests

The authors declare that they have no competing interests.
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