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REVIEW

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Comprehensive insight into endothelial progenitor cell-derived extracellular vesicles as a promising candidate for disease treatment

Ke Chen†, Yang Li†, Luwei Xu†, Yiguan Qian, Ning Liu, Changcheng Zhou, Jingyu Liu, Liuhua Zhou, Zheng Xu, Ruipeng Jia* and Yu-Zheng Ge*

Abstract

Endothelial progenitor cells (EPCs), which are a type of stem cell, have been found to have strong angiogenic and tissue repair capabilities. Extracellular vesicles (EVs) contain many effective components, such as cellular proteins, microRNAs, messenger RNAs, and long noncoding RNAs, and can be secreted by different cell types. The functions of EVs depend mainly on their parent cells. Many researchers have conducted functional studies of EPC-derived EVs (EPC-EVs) and showed that they exhibit therapeutic effects on many diseases, such as cardiovascular disease, acute kidney injury, acute lung injury, and sepsis. In this review article, we comprehensively summarized the biogenesis and functions of EPCs and EVs and the potent role of EPC-EVs in the treatment of various diseases. Furthermore, the current problems and future prospects have been discussed, and further studies are needed to compare the therapeutic effects of EVs derived from various stem cells, which will contribute to the accelerated translation of these applications in a clinical setting.

Keywords: Endothelial progenitor cells, Extracellular vesicles, Disease treatment, Cell communication, Comprehensive review

Introduction

In recent years, many research studies have revealed the different roles of stem cells and proposed their applications in the treatment of various diseases. Among the various classes of stem cells, which mainly include embryonic, haematopoietic, mesenchymal, and neural types, one class named endothelial progenitor cells (EPCs) has specifically attracted interest. Initially discovered by Asahara et al. in 1997, EPCs can be recruited to ischaemic tissue sites where they enhance collateral vessel growth [1]. EPCs can differentiate into mature endothelial cells (ECs) and directly participate in angiogenesis and revascularization [2]. Further studies have revealed that EPCs are involved in the repair and regeneration of damaged tissues [3–5]. Specifically, EPCs were found to be involved in the regeneration of ischaemic organs, playing important roles in the treatment of ischaemic brain injury [6] and repair of ischaemic renal tissue [7]. Additionally, the results from animal experiments and clinical studies have revealed that EPC-mediated therapy alleviates pulmonary arterial hypertension [8].

Extracellular vesicles (EVs), which are small intraluminal vesicles derived from different types of cells, are involved in the transport of endocellular contents such as cellular proteins, microRNAs (miRNAs), messenger RNA (mRNA), and long noncoding RNAs (lncRNAs) to the cell exterior [9]. EVs were previously described as

†Ke Chen, Yang Li, and Luwei Xu have contributed equally to this study

*Correspondence: ruipengj@163.com; geyuzheng@njmu.edu.cn

Department of Urology, Nanjing First Hospital, Nanjing Medical University, No. 68 Changle Road, Nanjing 210006, Jiangsu, People's Republic of China

"garbage bags" used by mature reticular cells to dispose of transferrin receptors [10], but their potential as carriers of intercellular communication is gradually being revealed [11]. Originating from bone marrow (BM), EPCs circulate in the peripheral blood (PB) and then migrate to the sites of both pathological and physiological angiogenesis [12]. EPC transplants were shown to stimulate angiogenesis by triggering angiogenic events or by differentiating into mature ECs [2]. However, EPC transplantation has some disadvantages, such as the potential for immunogenicity, malignant transformation, and embolus formation [13]. Most cell types, including EPCs, can release EVs [14]. Recent lines of evidence have demonstrated that stem cells likely achieve their effects through exosome secretion [15]. Some researchers have focused on EPC-derived EVs (EPC-EVs) because they are easier to manipulate than EPCs and have a stronger effect on skin wound healing and angiogenesis [16]. Moreover, due to the specific biological structure and features of these cells, anaphylactic reactions to EPC-EVs and their rejection by the body are seldom reported [17]. In this review article, we summarize the relationships between EPC-EVs and various diseases and then discuss current problems and future prospects related to their use for disease treatment.

Biogenesis and function of EPCs

EPCs are mainly derived from BM, PB, or cord blood (CB) and usually selected on the basis of certain cell surface antigen markers, such as vascular endothelial growth factor receptor 2 (VEGFR-2), CD34, and CD133 [18]. Additionally, culture and colony assays can be used to isolate EPCs [19]. These cells are usually classified as early or late EPCs according to their biological properties and culture time. Early EPCs appear after 5–7 days of culture and have a low proliferation rate, whereas late EPCs derived from mononuclear cells appear after 14–21 days of culture and have a high proliferative potential [20].

As presented in Fig. 1, EPCs repair damaged vessel walls through four steps: mobilization, homing, invasion, and differentiation/paracrine effects [21]. First, ischaemia promotes the transcription of molecules related to angiogenesis, such as adhesion molecules on ECs and vascular endothelial growth factor (VEGF), the latter of which is known to mobilize EPCs for differentiation [22]. Injured vasculature also induces EPC mobilization via other cytokines [23]. Once in the bloodstream, EPCs are affected by the concentration of chemokines and home to the vascular injury site. Upon reaching the site where the chemotactic agent is produced, the EPCs attach to the activated ECs of the damaged blood vessel wall via adhesion molecules and then enter the extracellular matrix

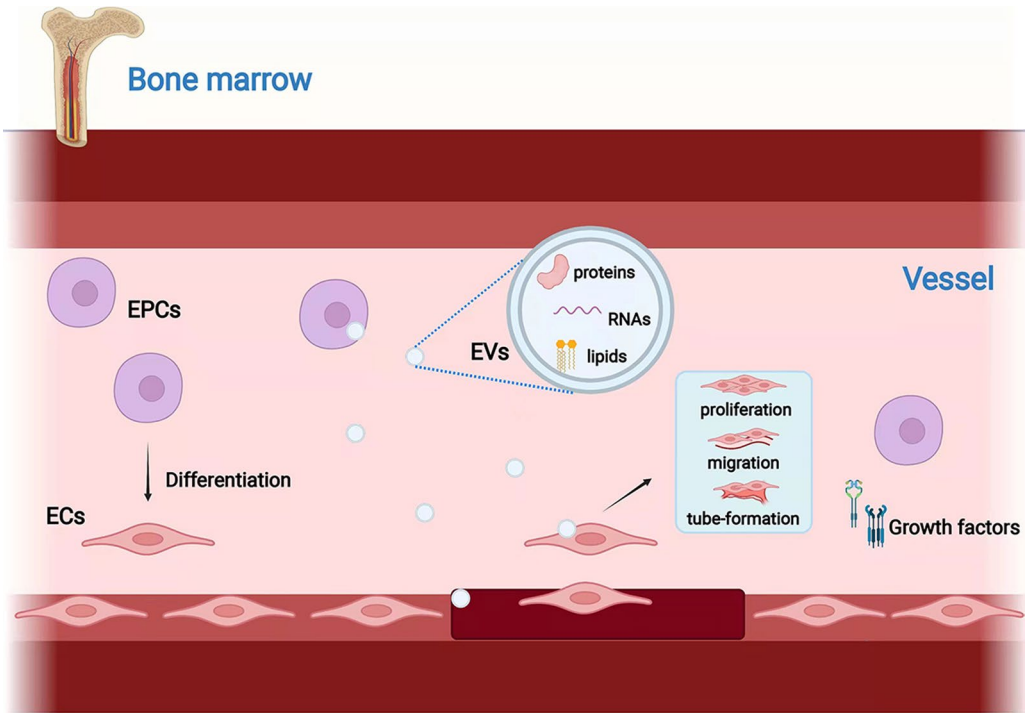

Fig. 1 Biogenesis and function of EPCs. Bone marrow-derived EPCs can differentiate into ECs; or stimulate the proliferation, migration, and tube formation of ECs through release of EVs, which contain proteins, RNAs, and lipids. (Created with BioRender.com)

from the vascular lumen via the endothelial intima, where they undergo differentiation into ECs. Additionally, EPCs can initiate vascular repair through paracrine signalling [24].

Biology of EVs

Biogenesis and release of EVs

EVs are produced in the form of apoptotic bodies (500–2,000 nm in diameter), exosomes (50–150 nm), and microvesicles (100–1,000 nm) [25], with the exosomes being distinguished from the other two EV types on the basis of surface protein expression and mode of biogenesis [26] (Fig. 2A). EVs are characterized by specific markers, such as membrane proteins (CD9, CD63, and CD81), major histocompatibility complex (MHC), Alix, TSG101, and HSP70 [27]. Exosomes also contain other proteins, DNA, and different types of RNA including miRNAs, mRNAs, and lncRNAs [28]. The processes of EV formation are complicated. In general, early endosomes are formed by cell membrane invagination, after which they will transform into late endosomes. Multivesicular bodies containing intraluminal vesicles are formed by inward budding of the limiting membrane, whereupon some are transported for cargo degradation in the lysosomes, some are transferred to the trans-Golgi network, and some release their intraluminal vesicles extracellularly as exosomes through fusion with the plasma membrane [25, 28, 29]. Target cells absorb EVs by endocytosis and fusion. Receptors on the membrane can facilitate EV uptake and regulate the cell signalling pathway [28, 30]. Although microvesicles have higher densities than exosomes, both types of EVs may overlap in size [31]. Microvesicles are formed through outward budding and fission of the plasma membrane and then shed [32]. Apoptotic bodies, the largest of the three types of EVs, contain nuclear material, organelles, membranes, and cytosolic content. Apoptotic bodies are released during the late stage of cell death through the membrane blebbing of apoptotic cells, a process induced through the phosphorylation of myosin light chain by Rho-associated kinase 1 (ROCK1), which in turn is activated by caspase-3 [33–35].

Isolation and characterization of EVs

EVs can be isolated by several different methods (Fig. 2B). Differential centrifugation is a classic strategy. EVs are separated by gradually increasing centrifugation time and

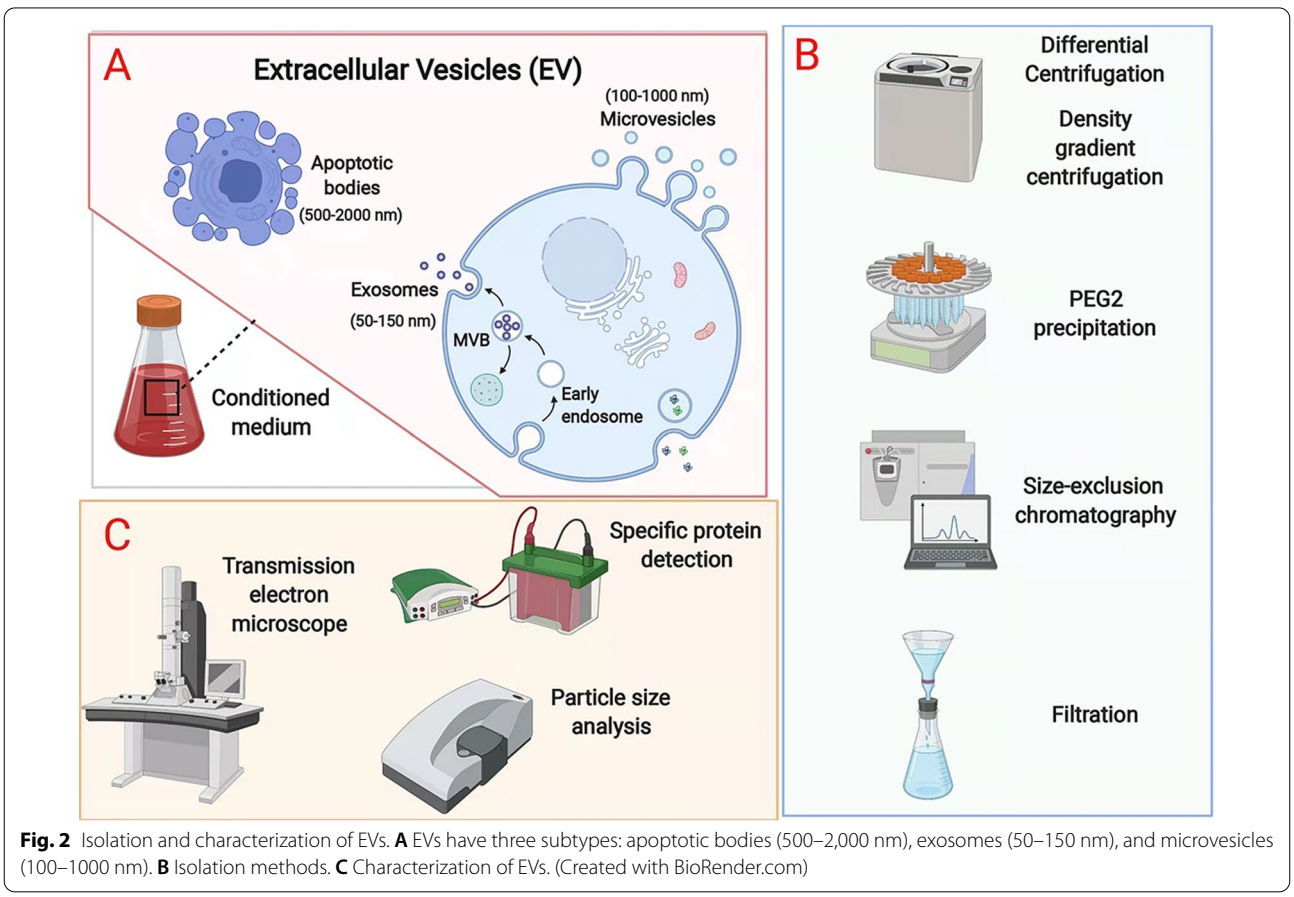

Fig. 2 Isolation and characterization of EVs. **A** EVs have three subtypes: apoptotic bodies (500–2,000 nm), exosomes (50–150 nm), and microvesicles (100–1000 nm). **B** Isolation methods. **C** Characterization of EVs. (Created with BioRender.com)

centrifugal force according to the different sedimentation rates of protein molecules, cells, and cell fragments in uniform suspensions [36]. Density gradient centrifugation is based on density differences in sample components, causing them to move to different locations [37]. The most widely used method is ultracentrifugation, which is relatively rapid, has a high efficiency, and results in a high purity [38]. However, there is no standard method for isolating EVs by centrifugation. PEG can also be used to change the solubility of EVs for precipitation because it can bind to the hydrophobic lipid bilayer due to its size and properties [39]. This method has the advantages of few devices needed, a high yield rate, and simple operation, but the purity of the EVs is low [40]. EVs can be differentiated on the basis of size, such as with ultrafiltration and size exclusion chromatography (SEC). The former method results in an unsatisfactory purity [41], while the latter method is time-consuming [42]. Having been widely used to separate biopolymers, gel filtration can be applied to isolate EVs [43], but this method is limited because it requires pretreatment to concentrate the EV samples [44]. Other approaches, such as methods involving lectins, antibodies, and lipid-binding proteins, use intermolecular interactions to capture EVs. Although their products are highly pure, these materials are expensive and difficult to process in large quantities [45].

As shown in Fig. 2C, transmission electron microscopy (TEM) and scanning electron microscopy can evaluate the morphology and structure of EVs [46]. Since the sizes of most of them are smaller than the minimum optical resolution, electron microscopes are the only way to visualize EVs [47]. Nanoparticle tracking analysis (NTA) can measure the particle concentration and size distribution [48] and occasionally the zeta potential [49]. In most studies, Western blotting (WB) is adopted to detect the surface markers of EVs [50] as WB can reveal the presence and amount of target proteins [51].

Potential biological functions of EVs

Emerging evidence has shown that EVs can act as biomarkers and therapeutic targets for various diseases. Additionally, these molecules play a vital role in cell communication.

Biomarker function

EVs are detectable in various bodily fluids, such as urine [52], blood [53], breast milk [54], and saliva [55]. They carry specific molecules from the parental cells and can also reflect current disease status. For example, microvesicles derived from human nipple aspirate fluid and blood are considered sources of nonintrusive molecular biomarkers for the early detection of various cancer types [56]. In the development of methods for EV detection, researchers have demonstrated that target membrane proteins can be used. Logozzi et al. was the first to develop a new enzyme-linked immunosorbent assay for detecting Rab-5b/caveolin-1 double-positive EVs in melanoma patients [57]. Shao et al. developed a rapid and sensitive analytical microfluidic chip platform that can distinguish patients with glioblastoma multiforme from healthy individuals [58]. The same research group also developed a high-throughput screening method that distinguishes ovarian cancer patients with a high degree of accuracy by targeting epithelial cell adhesion molecule (EpCAM) and CD24 on EVs in ascites [59]. Overall, EV-associated proteins can be used for disease detection, as they are more likely to be cancer related.

RNA is another important molecule found within EVs. Valadi and colleagues were the first to propose that mRNA- and miRNA-containing EVs might exert specific functions in recipient cells [11]. According to Ogata-Kawamata et al., colorectal cancer patients have high levels of seven miRNAs, which are decreased after tumour resection [60]. Matsumura et al. suggested that miR-19a-3p carried in EVs could serve as a prognostic biomarker to predict the recurrence of colorectal cancer [61]. The detection of EV-related RNAs identifies a novel biomarker strategy for cancer diagnosis and prognosis.

Cell communication

Accumulating lines of evidence have indicated that cell communication occurs through paracrine and endocrine signalling pathways (Fig. 3). Raposo et al. was the first to discover that antigen-specific T cell responses were induced by B cell-derived EVs with functional MHC II peptide complexes [62]. Samuelson et al. summarized the effect of EVs on cell-to-cell communication in metabolic regulation [63], and a similar role in exercise-induced adaptations was also reported [64]. According to Capra et al., EVs play important roles in various cell communication aspects, such as embryo–maternal crosstalk and oocyte maturation, fertilization, and implantation [65].

In cancer patients, EVs were shown to mediate tumour-to-stroma, stroma-to-tumour, and tumour-to-tumour communication [66]. EVs secreted by tumours can modulate the tumour microenvironment [66], thereby establishing cell communication pathways to support tumorigenesis [67]. The induction of tumour-promoting stroma by tumour-related EVs has been demonstrated [68] in prostate cancer, osteosarcoma, breast cancer, and colorectal cancer cells [69–72]. Conversely, stroma-secreted EVs can promote the growth, invasion, and metastasis of tumour cells. For example, Richards et al. demonstrated that EVs derived from cancer-associated fibroblasts enhanced the Snail protein level in pancreatic ductal adenocarcinoma cells, promoting their

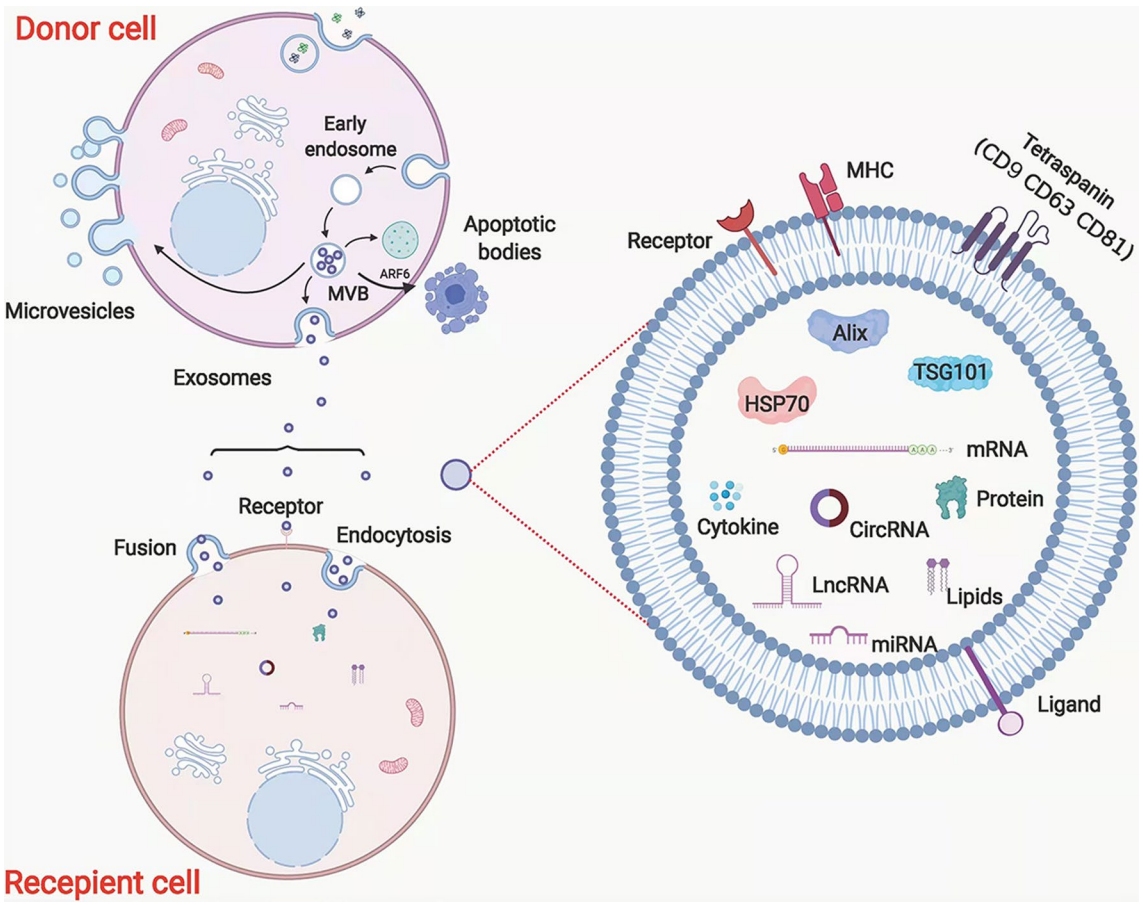

Fig. 3 EVs function as cell communication. Target cells absorb EVs by endocytosis and fusion, while receptors on the membrane can facilitate EVs uptake and regulate cell signal pathway. EVs are characterized by specific markers, such as membrane proteins (CD9, CD63, CD81), major histocompatibility complex (MHC), Alix, TSG101, and HSP70. (Created with BioRender.com)

proliferation and drug resistance [73]. The EV-mediated transfer of cancer cell-derived signals may also exert vital functions in different tumour cell subpopulations. For example, Al-Nedawi and colleagues showed that glioblastoma-derived EVs transferring the oncogenic EGFRvIII receptor led to oncogenic signalling activation in recipient tumour cells [74].

Therapeutic targets

To date, three types of EV-targeting therapeutic strategies have been proposed [75]. One of these is to eliminate the secretion of EVs, given that the molecules they carry can disturb cancer therapeutics and contribute to tumour progression, as reported for human epidermal growth factor receptor 2 (HER-2) located on EVs [76]. In this regard, Marleau et al. have already developed a system that can target HER-2 to bind cancer cell-derived EVs [77]. Chen et al. discovered that programmed death-ligand 1 (PD-L1), which acts in concert with programmed cell death protein 1 (PD-1) to suppress the antitumor immune response, was enriched in cancer-derived EVs and thus proposed the use of exosomal PD-L1 as a novel predictive biomarker for anti-PD-1 therapy [78]. Additionally, circulating EVs can be inhibited. For example, the neutral sphingomyelinase inhibitor GW4869 is widely used to inhibit both the release of EVs and the formation of intraluminal vesicles [79]. Blocking EV uptake is another way to suppress tumour progression, as shown in a study that used proteinase K to decrease the absorption of EVs by cancer cells [80]. All these studies demonstrated that EV-targeted therapy may be a new strategy for cancer treatment.

Potential roles of EPC-EVs in the treatment of various diseases

EPCs are widely considered a possible cell therapy source to promote tissue repair. However, the use of EPCs presents some concerns, such as ethical issues, cellular rejection, infusion toxicity, ectopic tissue formation, and possible tumorigenicity [81]. Therefore, EPC-EVs

have emerged as novel alternatives other than EPCs. As summarized in Additional file 1: Table S1 and Additional file 2: Table S2, EPC-EVs carry multiple common exosome markers, such as CD9, CD63, and CD81 [82], and some EPC-specific molecules including VEGFR-2, CD133, and CD34. Since the initial discovery of EPC-EVs, they have exhibited better therapeutic efficacy for various ischaemic diseases than traditional treatments. With continuing research, EPC-EVs have been shown to have various therapeutic roles in other diseases. In the following sections, we discuss the potent role of EPC-EVs in the treatment of various diseases and the current problems in their application as well as future perspectives (Additional file 1: Table S1).

Role in treatment of kidney disease

Under most circumstances, acute kidney injury (AKI) is caused by ischaemia–reperfusion injury (IRI) [83]. Recent studies, including ours, have shown that renal IRI can be repaired by EPCs through differentiation into ECs or endocrine/paracrine pathways [84–90]. Given these findings, Cantaluppi et al. postulated that EPC-EVs can exert a protective effect in AKI [91]. They found that EPC-EVs were localized within tubular cells and peritubular capillaries when injected following ischaemia–reperfusion. This process enhanced tubular cell proliferation and reduced leukocyte infiltration and cell apoptosis, which in turn conferred morphological and functional protection against AKI. In addition, EPC-EVs prevented the progression of chronic kidney damage by inhibiting glomerulosclerosis, capillary thinning, and tubulointerstitial fibrosis. Mechanistically, the renoprotective effect of EPC-EVs was exerted via transferring miR-126 and miR-296. AKI can also be induced by sepsis [92], a serious condition caused by a dysfunctional inflammatory response to infection [93]. Using a mouse model of sepsis-induced AKI mediated by lipopolysaccharide (LPS) treatment, He et al. found that EPC-EVs could inhibit apoptosis and inflammation by transferring miR-93-5p. In addition, miR-93-5p was found to directly target and inhibit KDM6B, induce H3K27me3, and inhibit TNF-α activation, thereby attenuating cell injury [94]. In another mouse model of sepsis-induced AKI constructed with caecal ligation and puncture (CLP) treatment, EPC-EVs were demonstrated to alleviate sepsis-induced AKI by releasing miR-21-5p to silence Runt-related transcription factor 1 (RUNX1). In the in vivo experiments, the researchers found reduced tubular degeneration and monocyte infiltration in the rats treated with miR-21-5p [95].

Glomerulonephritis, an inflammatory disease that affects the filtration of the glomeruli, can cause progressive fibrotic damage and chronic renal failure [96]. Cantaluppi et al. discovered that EPC-EVs could reduce proteinuria, suppress mesangial cell activation, leucocyte infiltration, and apoptosis, and inhibit glomerular injury by transporting specific RNAs. However, they did not determine the effective component of EPC-EVs [97]. In a follow-up study, these researchers demonstrated that EPC-EVs could protect the integrity of the glomerular filtration barrier from cytokine- and complement-induced injury, indicating that they may have a role in glomerulonephritis treatment [98]. Using a mouse model, Yang et al. showed that EPC-EVs could decrease pericyte–myofibroblast transition in renal fibrosis, thereby attenuating the injury [99].

Role in treatment of lung disease

Acute lung injury (ALI) is characterized by the infiltration of neutrophils in the alveolar–capillary barrier [100]. During ALI, inflammation causes sustained injury to the capillary endothelial barrier, resulting in pulmonary oedema, increased pulmonary vascular permeability, and hypoxemia [101]. With an ALI animal model generated via intratracheal administration of LPS, Wu et al. observed that histopathological ALI changes were significantly weakened, shown as reduced interstitial oedema, bleeding, alveolar wall thickness, and neutrophil infiltration in the lung parenchyma and alveolar space, and the arterial blood PaO_2 was improved in the group treated with EPC-EVs [102]. Through further in vitro experiments, the authors found that the knockdown of miR-126 inhibited the phosphorylation of RAF and extracellular signal-regulated kinases 1/2 (ERK1/2), whereas the EPC-EV transfer of this miRNA into target cells led to the downregulation of Sprouty-related Ena/vasodilator-stimulated phosphoprotein homology-1 domain 1 (SPRED1) expression and promotion of the RAF/ERK1/2 signalling pathways, which subsequently improved the function of ECs [102]. Similarly, using a combination of next-generation sequencing, an ALI mouse model, and in vitro transfection assays, Zhou et al. proved that EPC-EVs containing miR-126 could reduce the damage of ALI, whereas NIH3T3 cell-derived EVs carrying little miR-126 could not [103].

Role in treatment of bone disease

Long-bone defects are common conditions presented by patients in orthopaedic departments. For patients with long-bone defects attributed to postsurgical infections and complications or trauma, distraction osteogenesis (DO) is the primary treatment option [104]. Although DO can induce neo-osteogenesis, it requires a long consolidation period and has an increased risk of subsequent complications [105]. Jia et al. evaluated the effect of EPC-EVs in a rat model of unilateral tibial DO; bone

regeneration was strongly accelerated, as shown by histological, X-ray, and micro-computed tomography analyses in the EPC-EVs-treated group [106]. Additionally, the EPC-EV group had a higher vessel density than the control group. The results demonstrated that EPC-EVs strengthened EC migration, proliferation, and angiogenesis in a miR-126-dependent manner. Qin and Zhang observed that BM stromal cells treated with EPC-EVs showed decreased calcium deposition but increased colony-forming unit fibroblasts. The results indicated that EPC-EVs suppressed the expression of osteogenic genes and increased the proliferation of BM stromal cells, thereby regulating their osteoblastic differentiation in vitro [107]. Cui and colleagues proved that lncRNA-MALAT1 contained in EPC-EVs could promote bone repair by enhancing the recruitment and differentiation of osteoclast precursors [108].

Degenerative osteoporosis is usually a common issue in the elderly. However, the incidence of steroid-induced osteoporosis (SIOP) among younger individuals has shown an increasing trend [109]. There remain no effective treatment options for SIOP to date, simple and feasible therapies for this condition are urgently needed. Lu et al. established a mouse model of SIOP using high-dose dexamethasone and demonstrated through histopathological analysis that EPC-EVs treatment could increase the density and volume of the BM and trabecular bone [110]. Using Kyoto Encyclopedia of Genes and Genomes (KEGG) mapping, the authors further found that EPC-EVs partly reversed injury-induced changes in the ferroptosis pathway. Moreover, EPC-EVs reduced the dexamethasone-induced alterations in several oxidative injury markers.

Role in treatment of neurological injury

Stroke, a common ischaemic disease, is caused by the accumulation of inflammatory cells and the release of inflammatory factors due to local vascular tissue hypoxia and ischaemia, leading to local vascular EC necrosis and apoptosis [111]. Ischaemic stroke occurs when blood flow to the brain is disrupted. Restoration of flow or reperfusion can reduce injury but must be performed very early after ischaemia occurs [112]. Reactive oxygen species (ROS) and nitrogen are produced in the ischaemic penumbra during ischaemia and reperfusion [113]. Although ROS-induced vascular EC injury is known to play an important role in IRI, effective strategies to resolve this condition are lacking [114]. Wang et al. studied the relationship between hypoxia–reoxygenation (HR) injury and EPC-EVs in human brain microvascular ECs (hbECs). The authors concluded that EPC-EVs elicited their effects by regulating ROS production and the phosphatidylinositol 3-kinase (PI3K)/endothelial nitric oxide synthase (eNOS)/nitric oxide (NO) pathway. They also found that caspase-3 and miR-126 were delivered to hbECs by EPC-EVs. Unfortunately, the researchers did not further explore the underlying mechanism [115]. Similarly, Ma et al. overexpressed miR-210 in EPC-EVs, which resulted in significant reductions in HR-induced angiogenic dysfunction, EC apoptosis, and ROS production. However, these researchers could not rule out the influence of downstream functional targets, such as Efan3, Ptp1b, ISCU, and COX10 [116]. Li et al. found that miR-137 had neuroprotective effects against mitochondrial dysfunction and apoptosis, which might be dependent on the miR-137-cyclooxygenase 2 (COX2)/prostaglandin E2 (PGE2) signalling pathway [117].

Amyotrophic lateral sclerosis (ALS) is a serious neurological disease that can also cause cardiovascular failure [118]. Garbuzova-Davis et al. transplanted EPCs intravenously into a symptomatic superoxide dismutase 1 $(SOD1)^{G93A}$ mouse model of ALS to replace their damaged ECs, successfully restoring the blood–brain barrier [119, 120]. Subsequently, the same research group demonstrated that EPC-EVs could reduce mouse brain EC damage, identifying a new cell-free treatment for endothelial repair in ALS [121]. However, whether the protective effects of EVs on damaged cells are durable and whether cellular damage is reversible remain unclear. Furthermore, these in vitro results require EV administration to a mouse model of ALS in vivo to confirm the endothelial repair effect of the EPC-EVs.

Role in treatment of myocardial infarction

Myocardial infarction (MI) is one of the main causes of mortality and morbidity worldwide [122]. Cardiac fibroblasts are crucial for cardiac cell proliferation, angiogenesis, and cardiac tissue homeostasis and remodelling [123]. Ke et al. discovered that human EPC-EVs could increase angiogenesis and proliferation in cardiac fibroblasts by decreasing the expression of high mobility group box 1 protein B1 (HMGB1) and promoting mesenchymal–endothelial transition [124]. Further research proved that treatment with Exo-miR-363-3p or Exo-miR-218-5p improved the MI induced by left coronary artery ligation and recovered the integrity of the myocardial tissue. Compared with that of the control group, collagen expression was downregulated, and the degree of myocardial fibrosis was reduced in the EPC-EVs group. However, there are still some problems to be solved, such as the localization of miRNAs in EPC-EVs and identification of more potential miRNAs. Moreover, the role of EPC-EVs in the IRI model should be explored in the future, as the animal model in this study was a non-IRI model [125]. Huang et al. manipulated EPC-EVs with miR-1246 or miR-1290 and found that the upregulated

expression of either miRNA could promote phenotypic changes of fibroblasts to ECs and angiogenesis in cardiac fibroblasts, whereas their downregulation produced the opposite effects. However, the phenotype changes should be interpreted with caution as more markers are needed to characterize fibroblasts and ECs [126]. Yue et al. used IL-10-knockout (KO) mice to mimic inflammation and then compared the protein levels and therapeutic effect of exosomes derived from IL-10-KO-EPCs and wild-type EPCs (WT-EPCs). WT-EPC-Exo treatment strongly suppressed cell apoptosis, decreased MI scar size, improved left ventricular cardiac function, and facilitated post-MI neovascularization, whereas IL-10-KO-EPC-Exo treatment produced the opposite effects [127]. Liu et al. isolated circulating exosomes from mice with streptozotocin-induced diabetes for use in in vivo and in vitro experiments and concluded that exosomal miR-144-3p may hinder the mobilization of EPCs, which was related to the neovascularization damage induced by ischaemia, suggesting a novel strategy to improve cardiac repair after MI by intervening in the enriched miR-144-3p [128].

Some studies have demonstrated the consistency of the therapeutic effect of cardiac transplant cells without engraftment, indicating that paracrine mechanisms could potentially be used for therapeutic effects [129, 130]. Chen et al. delivered EPC-EVs within a shear-thinning gel to facilitate their exact localization and continuous delivery and succeeded in reproducing the advantageous effects of EPC treatment. In vivo studies showed that the delivery of EVs within the shear-thinning gel led to preservation of the ventricular geometry, enhanced peri-infarct vascular proliferation, and improved haemodynamic function after MI, thereby increasing the EV-mediated myocardial preservation effect. Nevertheless, translating shear-thinning gel as an EV-delivered tool to treat acute MI still requires more animal studies to test this approach [131]. Similarly, Chung et al. used a shear-thinning gel to deliver EPC-EVs and proved that such delivery at 4 days after MI preserved the holistic ventricular geometry and improved left ventricular contractility [132].

Role in treatment of non-MI cardiovascular injury

The incidence of cardiovascular disease continues to increase annually worldwide. Vascular endothelial injury may cause potential changes, such as thrombosis, inflammation, and smooth muscle cell (SMC) proliferation, leading to neointimal hyperplasia, unfavourable arterial remodelling, and restenosis [133]. Using a rat model of vascular injury, Li et al. found that EPC-EVs accelerated re-endothelialization at an early stage after injury, and in vitro analyses indicated that this treatment could strengthen the proliferation and migration of ECs [134]. SMC proliferation is believed to be a key factor for restenosis following endothelial injury [135]. In a study by Kong et al. on the effects of exosomes on SMCs and ECs, in vivo assays showed that the intimal-to-medial area ratio was significantly reduced and SMC proliferation was significantly lower in the exosome group than in the control group [136]. The in vitro study confirmed that the administration of exosomes could significantly enhance the migration and proliferation of SMCs and ECs, indicating that EPC-EVs likely inhibited neointimal hyperplasia in the rat model through the promotion of EC repair. Hu et al. proved that EPC-EVs were more efficacious than EC-EVs for vascular repair [137]. In another study, Hu et al. investigated the mechanism of EPC-EVs in endothelial repair and concluded that the exosomal delivery of miR-21-5p may promote EC repair by inhibiting thrombospondin 1 (THBS1). However, the researchers could not rule out the effects of other EPC-EVs in EC repair. Therefore, further experiments are required to rule out the interference of other miRNAs [138]. Recently, EPCs expressing the bone matrix protein osteocalcin were found to be related to the severity of cardiovascular diseases [139]. Subsequently, Yi et al. proved that overexpressed osteocalcin (OCN) in EPCs had beneficial effects on EC proliferation, migration, and function through the exosomal pathway, participating in the promotion of angiogenesis and NO formation via the interaction of OCN and its receptor G protein-coupled receptor family C group 6 member A (GPRC6A). Due to the lack of specific receptor antagonist of GPRC6A and the fact that OCN and GPRC6A are not specific to each other, the findings should be verified with further well-designed studies [140]. Cardiovascular homeostasis is regulated by the renin–angiotensin system, in which angiotensin II (Ang II) is the main peptide and is related to vascular dysfunction [141]. Wang et al. suggested that EPCs could repair EC injury through their exosomal effects on mitochondrial function and angiotensin-converting enzyme 2 (ACE2) overexpression [142]. These researchers subsequently proved that ACE2 enhanced the effects of EPC-EVs on the Ang II-induced inhibition of vascular SMC phenotypic modulation by restraining nuclear factor-kappa B (NF-κB) signalling [143].

Cardiovascular diseases can be caused by many other diseases, such as bronchopulmonary dysplasia (BPD), a severe lung disease in extremely preterm infants [144]. Using an experimental model of BPD obtained by exposing pulmonary microvascular ECs to hyperoxia, Zhang et al. proved that the administration of EPC-EVs enhanced the bioactivity of ECs in vitro and increased the expression levels of VEGF, VEGFR-2, and eNOS relative to those in the untreated hyperoxia group. However, the exact carriers of EPC-EVs and the molecular

mechanisms remain yet to be explored, and the in vivo studies are warranted to validate the ex vivo findings [145]. Atherosclerosis is a chronic inflammatory disorder characterized by endothelial dysfunction. Li et al. proved that EPC-EVs can suppress the ferroptosis of ECs and mitigate the occurrence of atherosclerosis by transferring miR-199a-3p to inhibit specificity protein 1 (SP1) [146].

Role in treatment of sepsis

Sepsis, which is a dysfunctional systemic inflammatory disease caused by infection, usually leads to organ failure and even death [147]. Previous studies have shown that EPCs have beneficial effects on organ dysfunction, vascular injury, and mortality in sepsis models [148]. Based on the assumption that EPC-EVs can transfer miRNAs to protect the microvasculature, Zhou et al. used the CLP method to generate a mouse model of sepsis to test their hypothesis, and EPC-EVs treatment improved the survival rate of septic mice, inhibited renal and lung vascular leakage, and reduced kidney and liver dysfunction. The sepsis-induced increase in plasma cytokine and chemokine levels was also attenuated by EPC-EVs. The investigators explored the genome-wide miRNA expression patterns in EPC-EVs and focused on one specific miRNA: miR-126. However, they could not rule out the influence of additional miRNAs, lipids, and proteins [149]. Similarly, Hong and colleagues discovered that EPC-EVs improved heart function by suppressing oxidative stress, inflammation, and apoptosis and attenuated the pathological damage of myocardial tissues in septic rats, providing a novel therapeutic strategy against myocardial damage in sepsis [150].

According to a whole-blood transcriptomic study, lncRNA taurine upregulated gene 1 (TUG1) is one of the top five sepsis-relevant lncRNAs [151]. TUG1 alleviated sepsis-induced inflammation and apoptosis by targeting growth factor receptor-bound protein 2 (GRB2)-associated binding protein 1 (GAB1) and miR-34b-5p [152]. Ma et al. explored the effect of EPC-EVs-delivered TUG1 in septic mice and demonstrated that miR-9-5p could bind competitively with TUG1, which upregulated the expression of sirtuin 1 (SIRT1) and promoted M2 macrophage polarization. In a mouse model, EPC-EVs carrying TUG1 were shown to reduce the organ damage induced by sepsis mainly through macrophage M2 polarization [153].

Role in treatment of diabetes

The global incidence of diabetes is increasing, and complications have become serious public health problems [154]. Approximately 20% of patients develop diabetic wounds, the most common being leg or foot ulcers [155], which can reduce physical activity, result in chronic ischaemic skin lesions, and even lead to limb amputations in serious cases [156]. Li et al. found that EPC-EVs could heal diabetic wounds in diabetic rats and enhance the proliferation, migration, and tube formation of vascular ECs in vitro. Moreover, ECs stimulated with these exosomes showed increased expression of angiogenesis-related molecules. However, this study focused on the phenotype assays without exploring the active ingredients in EPC-EVs [157]. Similarly, Zhang et al. discovered that the proliferation, migration, and tube formation of ECs could be enhanced by EPC-EVs. Furthermore, the exosomal treatment altered the expression of genes related to the ERK1/2 signalling pathway, which was the pivotal mediator during the EV-induced angiogenic responses of ECs by functional study confirmation [158]. Xu et al. treated diabetic mice bearing skin wounds with miR-221-3p, EPC-EVs, or phosphate-buffered saline and showed that wound healing was strengthened by EPC-EVs and miR-221-3p compared to that in both control and diabetic mice. In this study, EPC-EVs were directly spread onto wound sites, which was convenient and practical in the clinical settings [159].

Patients living with diabetes have a 3–4 times higher risk of suffering an ischaemic stroke than those without diabetes because the combination of decreased angiogenesis and impaired endothelial dysfunction aggravates cerebral damage [160]. Wang found that EPC-EVs protected ECs against HR-induced dysfunction and injury [115] and enhanced the function and viability of EPCs in diabetes [161]. In a subsequent study, these researchers found that EPC-EVs–miR-126 had better effects than EPC-EVs in increasing cerebral blood flow and microvascular density, decreasing the infarct size, and promoting neurogenesis and angiogenesis as well as neurological functional recovery. However, as different cell types including neurons, vascular ECs, and neuroblasts can express VEGFR-2, further research is needed to determine which brain cells are the main ones with increased VEGFR-2 expression [162]. Atherosclerosis is a main macrovascular complication of diabetes and the leading cause of death in patients with diabetes [163]. Bai et al. found that most of the top ten upregulated miRNAs in EPCs-derived exosomes were associated with atherosclerosis. Further in vivo studies showed that treatment with EPC-EVs could reduce the production of inflammatory factors and diabetic atherosclerotic plaques. Nonetheless, the exact miRNA in EPC-EVs that plays an important role in diabetic atherosclerosis remains unclear [164].

Future perspectives and conclusions

With the development of regenerative medicine and stem cell therapy, various stem cells have been proven to be effective in tissue damage repair [165, 166]. EPCs are one class of stem cells that are known to repair and

regenerate various tissues [81, 167] and have attracted increasing attention for their stem cell-specific functions as well as the regulatory function of their EVs. EPC-EVs have exhibited better effects than EPCs in some preclinical studies [131]. EVs are less immunogenic than their parental cells and can be transported and stored for a long time [168]. Moreover, aside from their abilities to deliver various molecules that regulate angiogenesis, fibrosis, and cell proliferation, EPC-EVs can be utilized as cell-free drug carrier systems, which have the advantages of availability and reproducibility [169]. The feasibility of loading or enriching EVs with specific regulatory molecules has been proven [170, 171], and the application of modified EPC-EVs as vehicles for delivering regulatory factors may elicit better therapeutic effects.

However, some issues should be addressed before the translational application of EPC-EVs in the clinical settings. Firstly, the differential therapeutic effects of EVs derived from various stem cells including EPCs remain to be determined. To date, various classes of stem cells and their EVs were applied in disease treatment, among which adipose-derived stem cells (ADSCs) and bone marrow stem cells (BMSCs) were studied extensively [172–175]. As summarized in Additional file 2: Table S2, the EVs from ADSCs and BMSCs exhibited similar characteristics and exerted protective functions in a wide array of diseases like EPC-EVs. Recently, Miyasaki and colleagues compared the efficiency of EVs from mesenchymal stem cells and EPCs in treating chronic kidney disease and found similar improvement in renal function in terms of serum albumin, cystatin C, crystals in the renal tubules, and fibrosis, rather than staining of alpha-smooth muscle actin [176]. However, the direct comparison between different types of EVs was rare, and further studies are needed.

Secondly, a consensus on the source, identification, and culture conditions of EPCs is needed. As stated before, EPC-EVs isolated from PB [91], CB [94], and BM [177] exhibited protective effect on kidney injury, through different mechanisms. The number of EPCs in BM (0.05%) and PB (0.01%) is relatively low [178], while CB-derived EPCs have a higher proliferative potential and higher frequency than other EPC types [179, 180]. Although positivity for CD133, VEGFR-2, and CD34 is widely accepted when referring to EPCs, two distinct classes (early or late EPCs) were reported with different combinations of defining markers, which needs standard protocols for the detection of EPCs [19]. Regardless of the cell source and identification process, the number of EPCs was too low for further applications, while ex vivo culture can contribute to the matureness of stem cells and a reduction in cell number [178]. Hence, the head-to-head comparative studies on EVs derived from EPCs with different cell source, definition, and culture conditions should be performed, which might conclude a consensus and guideline for the clinical application.

Thirdly, the exact effective molecules within EPC-EVs remain to be explored comprehensively. Most of the cited studies focused on the phenotype changes under EPC-EVs treatment, which lacked validation from different levels and detailed investigation of the specific active ingredients in EPC-EVs. Different components have been reported in EPC-EVs, such as miRNAs, lncRNAs, proteins (Additional file 1: Table S1), and mRNAs [181]. Among which, miRNAs have been explored widely, and most of the candidate molecule was selected without high-throughput screening. Mounting evidence has indicated the existence of lipids [182], circRNAs [183], and DNA [184] in the EVs derived from different stem cells, which remain yet unknown in EPC-EVs. Further well-designed studies with substantial verification are warranted to examine the effective components in a comprehensive and unbiased manner.

Lastly, the EV extraction process is complicated and has yet to be standardized. The quality standard of the entire production process must be medically satisfied and certified, which affects economic sustainability [185]. Importantly, the mass production of EPC-EVs is critical for clinical needs [186]. Moreover, the establishment of the dose and delivery route of EPC-EVs requires more research, as the amount and content of exosomal cargo are different under different pathophysiological conditions. Most studies simply analysed the amount and content of exosomes at a single time point and did not completely investigate all exosomal contents that were differentially expressed.

Because the complexity and high off-target rate of EVs are the main obstacles to clinical application, a new strategy for comprehensively simulating EVs is needed [187]. Although artificial EVs are easier to mass-produce and more uniform [188], additional research is needed in the preclinical setting. Hopefully, with the continued development of tissue engineering and nanotechnology, transporting EPC-EVs to the right place can be achieved, which may result in more effective treatment of different diseases. Given our limited understanding of EPC-EVs, their long-term therapeutic safety is difficult to predict. Therefore, extra care is required during the transition of this treatment strategy to the clinic.

Abbreviations

ADSCs: Adipose-derived stem cells; Ang II: Angiotensin II; ACE2: Angiotensin-converting enzyme 2; ALI: Acute lung injury; ALS: Amyotrophic lateral sclerosis; AKI: Acute kidney injury; BM: Bone marrow; BMSCs: Bone marrow stem cells; BPD: Bronchopulmonary dysplasia; CB: Cord blood; CLP: Caecal ligation and puncture; COX2: Cyclooxygenase 2; DO: Distraction osteogenesis; ECs: Endothelial cells; eNOS: Endothelial nitric oxide synthase; EpCAM: Epithelial

cell adhesion molecule; EPC-EVs: EPC-derived EVs; EPCs: Endothelial progenitor cells; ERK1/2: Extracellular signal-regulated kinases 1/2; EVs: Extracellular vesicles; GPRC6A: G protein-coupled receptor family C group 6 member A; GAB1: Growth factor receptor-bound protein 2 (GRB2)-associated binding protein 1; GRB2: Growth factor receptor-bound protein 2; hbECs: Human brain microvascular ECs; HER-2: Human epidermal growth factor receptor 2; HMGB1: High mobility group box 1 protein B1; HR: Hypoxia reoxygenation; IRI: Ischaemia–reperfusion injury; KEGG: Kyoto Encyclopedia of Genes and Genomes; KO: Knockout; lncRNAs: Long noncoding RNAs; LPS: Lipopolysaccharide; MiRNAs: MicroRNAs; mRNA: Messenger RNA; MI: Myocardial infarction; MHC: Major histocompatibility complex; NTA: Nanoparticle tracking analysis; NO: Nitric oxide; NF-κB: Nuclear factor kappa B; OCN: Osteocalcin; PB: Peripheral blood; PD-L1: Programmed death-ligand 1; PD-1: Programmed cell death protein 1; PI3K: Phosphatidylinositol 3-kinase; PGE2: Prostaglandin E2; ROCK1: Rho-associated kinase 1; ROS: Reactive oxygen species; RUNX1: Runt-related transcription factor 1; SEC: Size exclusion chromatography; SPRED1: Stimulated phosphoprotein homology-1 domain 1; SIOP: Steroid-induced osteoporosis; SOD1: Symptomatic superoxide dismutase 1; SP1: Specificity protein 1; SIRT1: Sirtuin 1; SMC: Smooth muscle cell; TEM: Transmission electron microscopy; THBS1: Thrombospondin 1; TUG1: Taurine upregulated gene 1; VEGF: Vascular endothelial growth factor; VEGFR-2: Vascular endothelial growth factor receptor 2; WB: Western blotting; WT-EPC: Wild-type EPCs.

Supplementary Information

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Additional file1: Table S1. The beneficial effects of EPC-EVs on various diseases.

Additional file2: Table S2. The beneficial effects of ADSC- and BMSC-EVs on various diseases.

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Author contributions
CK, LY, and XLW collected the literature and wrote the original draft. QYG, LN, and ZCC prepared the table. LY, LJY, ZLH, and XZ prepared the figures. JRP and GYZ conceived the idea and revised the manuscript. All authors have read and approved the final manuscript.

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Declarations

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