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STEM CELLS

The role of small extracellular vesicles in cerebral and myocardial ischemia—Molecular signals, treatment targets, and future clinical translation

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Abstract

The heart and the brain mutually interact with each other, forming a functional axis that is disturbed under conditions of ischemia. Stem cell-derived extracellular vesicles (EVs) show great potential for the treatment of ischemic stroke and myocardial infarction. Due to heart-brain interactions, therapeutic actions of EVs in the brain and the heart cannot be regarded in an isolated way. Effects in each of the two organs reciprocally influence the outcome of the other. Stem cell-derived EVs modulate a large number of signaling pathways in both tissues. Upon ischemia, EVs prevent delayed injury, promote angiogenesis, enhance parenchymal remodeling, and enable functional tissue recovery. The therapeutic effects greatly depend on EV cargos, among which are noncoding RNAs like microRNAs (miRNAs) and proteins, which modulate cell signaling in a differential way that not always corresponds to each other in the two tissues. Interestingly, the same miRNA or protein localized in EVs can modulate different signaling pathways in the ischemic heart and brain, which may have diverse consequences for disease outcomes. Paying careful attention to unveiling these underlying mechanisms may provide new insights into tissue remodeling processes and identify targets for ischemic stroke and myocardial infarction therapies. Some of these mechanisms are discussed in this concise review, and consequences for the clinical translation of EVs are presented.

KEYWORDS
adult human bone marrow, adult stem cells, bone marrow stromal cells, cell transplantation, mesenchymal stem cells, microRNA

1 | THE HEART-BRAIN AXIS UNDER ISCHEMIC CONDITIONS

Ischemic stroke and myocardial infarction are highly prevalent diseases with common pathophysiological mechanisms, which are leading causes of long-term disability and death world-wide.[1] Whereas previous studies examined these two diseases separately, recent knowledge resulted in building up a heart-brain axis concept, upon which the heart and the brain mutually interact with each other under physiological and pathological conditions.[2,3] Under physiological conditions, the central nervous system (CNS) maintains the heart rhythm through the sympathetic and the parasympathetic nervous systems.[4] Under pathological conditions such as ischemic stroke, the regulation of the heart rhythm by the brain is disrupted. Ischemic lesions of the insular cortex, for instance, cause severe cardiac arrhythmias and autonomic dysfunction.[5,6] Specifically, left hemispheric brain

infarction has been associated with an elevated risk of adverse cardiac outcomes and increased long-term mortality.[7] Ischemic stroke can induce severe cardiac dysfunction such as Takotsubo cardiomyopathy or neurogenic stress myocardiopathy associated with cardiac wall motion abnormalities.[2] As a matter of fact, the majority of patients suffering from acute ischemic stroke will develop at least one serious cardiac adverse event or left ventricular diastolic dysfunction during the first 3 months after stroke.[8,9] Severe heart disease, conversely, also affects the brain. Patients who experienced a heart failure are more likely to suffer from ischemic stroke or chronic brain hypoperfusion.[10] Of note, stroke incidence doubles in the presence of coronary heart disease and quadruples in the presence of heart failure.[11]

Mechanisms underlying heart-brain axis disturbances include central autonomic network disturbances, deregulated parasympathetic and sympathetic activity, hypothalamic-pituitary-adrenal axis deregulation with excessive catecholamine release, blood-brain barrier disruption, as well as local and systemic inflammatory responses.[3,12,13] Although several questions regarding the underlying mechanisms of heart-brain interactions under physiological and pathophysiological conditions remain to be identified, it is meanwhile established that therapeutic actions on one side of the axis also influence the other side as well. On the contrary, drugs that affect both ischemic organs directly and independent from each other might yield superior therapeutic effects. This aspect will be addressed more closely when discussing different signaling pathways that are regulated by extracellular vesicles (EVs).

2 | CELL-BASED REGENERATIVE CONCEPTS IN CEREBRAL AND MYOCARDIAL ISCHEMIA

Recanalization strategies have significantly been improved in recent years, contributing to clinically favorable outcomes after ischemic stroke and myocardial infarction.[14] However, many patients do not qualify for recanalization therapies because of narrow time windows, potential side effects, or contraindications.[15] Hence, novel treatment strategies are currently under investigation, among which stem cell transplantation has gained increasing interest.

Stem cell transplantation has successfully been applied after stroke and myocardial infarction in the last decade,[16-20] with different stem cell sources demonstrating great potential in clinical practice.[21,22] The use of stem cell-derived EVs has opened a new scientific field.[23] Compared with stem cells, EVs are easy to obtain and have few side effects, especially with regard to malignant transformation, which is an inherent risk of cell therapy. EVs are a heterogeneous group of lipid bilayer structures that are secreted from virtually all cells,[24] among which are exosomes and microparticles. Depending on the type of EV, their diameters range between 30 and 1000 nm. For purposes of simplicity, we will only use the term EVs in the present review. These EVs achieve their biological functions by various cargos such as RNAs, DNAs, and proteins.[25] As different stem cell phenotypes have been successfully applied in various disease models, so have been their corresponding EV secretion products. Indeed, EVs derived from neural progenitor cells (NPCs) or mesenchymal stem cells (MSCs) yield similar therapeutic effects when compared with their host cells.[26-28]

Significance statement

Stem cell-derived extracellular vesicles show great potential as a therapeutic tool in myocardial infarction and cerebral ischemia. However, the underlying mechanisms of stem cell-derived extracellular vesicles are still unclear. In this article, the authors summarize some mechanisms to be involved in stem cell-derived extracellular vesicles in stroke and in myocardial ischemia. The authors also briefly discuss common pathways that may be affected by stem cell-derived extracellular vesicles in both myocardial infarction and cerebral ischemia.

Despite similar effects of stem cell-derived EVs and stem cells, the EV contents are the critical biological factors. As such, Liu et al established a database that summarizes microRNA (miRNAs) located inside EVs from different cell sources, including MSCs.[29] However, Liu and colleagues did not further differentiate between various MSC subtypes. Herein, we will focus on EVs and their miRNA profiles from three commonly used human MSCs, that is, bone marrow-derived MSCs (BM-MSCs),[30] umbilical cord-derived MSCs (UC-MSCs),[31] and adipose tissue-derived MSCs (AD-MSCs).[32] Although there are only 11 common miRNAs in these MSC-EVs, the latter can induce many beneficial effects in various disease models. Unfortunately, research on murine MSC-EV miRNAs is sparse, and miRNA profiles of many MSC sources found in the peripheral blood, lung or dental pulp are still undetermined. It has been previously suggested that small EVs carrying miRNAs are exosomes. More recent studies separating small EVs based on GM1 ganglioside, globotriaosylceramide and phosphatidylserine contents via choleratoxin subunit b, shiga toxin subunit b, and annexin V binding have shown that at least three subtypes of small EVs can be defined.[33] Of these EVs only GM1 ganglioside-rich vesicles are bona fide exosomes. On the contrary, globotriaosylceramide-rich vesicles originate from the cell nucleus, whereas phosphatidylserine-rich vesicles have roles in waste and cell debris extrusion. Importantly, only nucleus-derived vesicles contained significant miRNA amounts.[33] A summary of miRNAs that are found in EVs derived from BM-MSCs, UC-MSCs, and AD-MSCs is given in Figure 1.

3 | STEM CELL-DERIVED EVs IN CEREBRAL ISCHEMIA

Although MSC transplantation appears to be safe,[34] intravascular administration can induce microvasculature occlusions due to the relatively large size of MSCs.[35] Since stem cell-derived EVs and their host

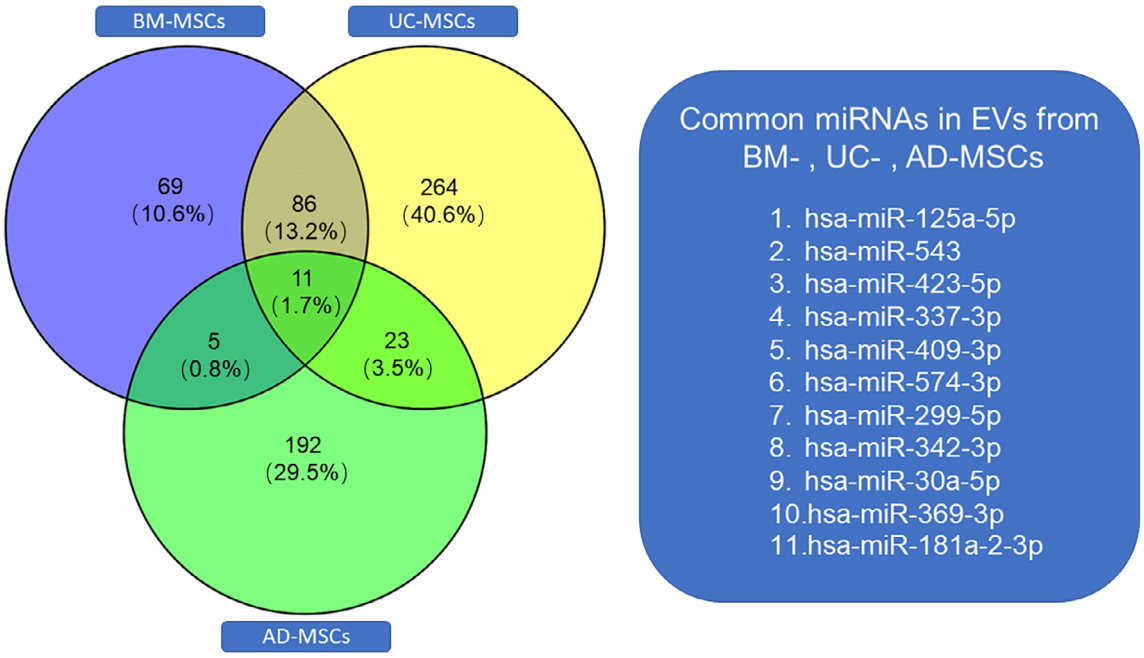

FIGURE 1 Common miRNAs found in extracellular vesicles (EVs) from different mesenchymal stem cell (MSC) sources. Prominent cell sources of EVs are bone marrow-derived mesenchymal stem cells (BM-MSCs), umbilical cord-derived mesenchymal stem cells (UC-MSCs), and adipose-derived mesenchymal stem cells (AD-MSCs). There are a total of 171 miRNAs in BM-MSCs, 384 miRNAs in UC-MSCs, and 231 miRNAs to be found in AD-MSCs. A number of 69 miRNAs are unique to BM-MSCs, 264 to UC-MSCs, and 192 are only found in AD-MSCs. BM-MSC-derived EVs and UC-MSC-derived EVs share 97 miRNAs. Compared with BM-MSCs, AD-MSCs display less common miRNAs with both UC-MSC-derived and BM-MSC-derived EVs (16 common miRNAs with BM-MSCs and 34 common miRNAs with UC-MSC)

cells yield similar results under physiological and pathological condition,[36] stem cell-derived EVs are now considered as clinically-applicable alternative to cell transplantation. MSC-derived EVs increase neurogenesis and angiogenesis along the infarct border in a rat focal cerebral ischemia model.[37] Moreover, MSC-derived EVs improve synaptic transmission, long-term potentiation and cognitive impairment after transient global cerebral ischemia in mice.[38] Previous work of our own group demonstrated that BM-MSC-EVs were not inferior to MSCs in a mouse focal cerebral ischemia model.[39] MSC-EV injection induced long-term neuroprotection, increased angiogenesis and neurogenesis, and enhanced motorcoordination recovery.[39] Interestingly, MSC-EVs reversed peripheral postischemic immunosuppression in the subacute stroke phase.[39] Postischemic immunosuppression is clinically relevant, since it predisposes stroke patients to lung infections and sepsis, which are a premier cause of patients' death.[40] The idea that restored immune responses may have contributed to the enhanced angiogenesis, neurogenesis, and neurological recovery in ischemic mice is promising. Blood-derived leukocytes, specifically polymorphonuclear neutrophils, play critical roles in postischemic extracellular matrix degradation,[41] a prerequisite for microvascular migration and sprouting. Similar results such as reduced brain injury, enhanced white matter integrity, and enhanced motorcoordination recovery were observed using human neural stem cell-derived EVs in a pig focal cerebral ischemia model,[42] suggesting that stem cell-derived EVs might have recovery-promoting effects independent of the cell source. Yet, major influences were attributed to the culture conditions, in which EVs were harvested. Thus, MSCs exposed to ischemic brain extracts were found to release EVs with restorative characteristics that are superior to nonmanipulated MSCs.[43] Although the optimal timing and delivery route for EVs is discussed, Otero-Ortega et al reported in a focal cerebral ischemia model in rats that single injections of MSC-EVs might be sufficient to induce functional motorcoordination recovery, preserve white matter integrity and increase axonal plasticity.[44] In their study, the effects of EVs were attributed to repair factors acting on oligodendrocytes, astrocytes, and neurons.

In view of their numerous targets, which they modify by posttranscriptional regulation, miRNAs located inside of EVs are potent repair factors. Previous work from our group in a model of focal cerebral ischemia in mice demonstrated that AD-MSC-EVs transfer miRNA-25, modulating autophagy in ischemic neurons by targeting BNIP3, which results in increased cell viability.[45] Likewise, Xin et al also reported in a model of focal cerebral ischemia in rats that BM-MSCs transfer miR-133b to neurons via EVs to increase neurite branch numbers and total neurite length under ischemic conditions.[46] The authors observed that miR-133b concentrations in EVs were increased when MSCs were pretreated with ischemic brain extracts in vitro before usage. Application of such MSC-EVs resulted in increased intracellular levels of miR-133b in astrocytes and neurons, suggesting that EVs might mediate miR-133b transfer to neurons and astrocytes. Increased levels of miR-133b, in turn, can downregulate CTGF (connective tissue growth factor) expression in astrocytes, which decreases poststroke glial scar

formation.[47] Notably, miR-133b also reduces RhoA protein expression,[48,49] which increases corticospinal tract plasticity after spinal cord injury.[49] Hence, miR-133b was suggested to be essential for neurite outgrowth and functional recovery.[50]

Zhang et al demonstrated that the miR-17-92 cluster in MSC-EVs could be one of the key players in promoting axonal growth.[51] Using a neuronal cell culture in a microfluidic device, they observed axonal growth after applying MSC-EVs. However, the proaxonogenesis effect was abolished after applying siRNA-Ago2 to MSC-EVs, which suggested that Ago2 (argonaute-2) was crucial for EV-promoted axonal growth. Interestingly, the miR-17-92 cluster was reduced in Ago2-reduced EVs. By elevating miR-17-92 clusters in EVs, MSC-EVs enhance their potential to further promote axonal growth.[52] At least some of these effects mediated by miR-17-92 can be attributed to a regulation of the PTEN (phosphatase and tensin homolog)/mTOR pathway within the distal axons of cortical neurons, leading to promote axonal growth.[52] Likewise, miR-138-5p located in BM-MSC-EVs reduces neurological impairment by promoting cell proliferation and inhibiting inflammatory responses within the ischemic milieu.[53] The miR-138-5p could promote proliferation and inhibit apoptosis of astrocytes by targeting the LCN2 (lipocalin 2) gene.[53] Another miRNA found in MSC-EVs, miR-let-7b, has been described to affect the TLR4 (toll-like receptor 4)/NF-κB/STAT3 (signal transducer and activator of transcription 3)/PKB (protein kinase B) pathway, resulting in induction of macrophage polarization in direction of an anti-inflammatory phenotype.[54] Inhibition of proapoptotic signaling cascades is also observed by miR-134 located in MSC-EVs, which improves oligodendrocyte survival by suppressing caspase-8.[55] Small EVs from human urine-derived stem cells can also induce neurogenesis by transferring miR-26a, as shown in a rat focal cerebral ischemia model.[56] This effect was partly attributed to miR-26a-induced inhibition of deacetylase 6.[56] On the contrary, previous research from our group and others have shown that MSC-EVs may rather affect peripheral organs instead of the CNS itself.[39] This is in line with supporting evidence showing that most EVs are trapped in peripheral organs such as the lung when choosing a systemic delivery route.[57] Such models use fetal bovine serum (FBS), limiting the translational process from preclinical settings to clinical application. However, the application of a standardized platelet lysate supplement might overcome these limitations.[58]

Beside miRNAs, proteins are enriched in EVs as well. Although there are relatively few studies on individual proteins in ischemic stroke models, proteomic analyses on EVs provided hints regarding potential signaling pathways modulated by EVs. In a model of mild focal cerebral ischemia in rats resulting in subcortical stroke, 2416 proteins regulated by MSC-EVs were discovered that are implicated in poststroke brain repair.[44] Many signaling cascades were found to be affected by EVs. Among these, the VEGF and STAT3 pathways are noteworthy, triggering poststroke angiogenesis and tissue regeneration in vitro and in vivo via mechanisms that involve the inhibition of autophagy.[59,60] The molecular targets and functions of MSC-derived miRNAs and proteins under conditions of cerebral ischemia are summarized in Table 1.

4 | STEM CELL-DERIVED EVs IN MYOCARDIAL ISCHEMIA

A meta-analysis indicated that stem-cell-derived EVs significantly reduce infarct size and improve cardiac function in an animal model of myocardial infarction.[61] EVs from different MSC sources were found to protect cardiomyocytes from injury and boost angiogenesis.[62-64] In a rat myocardial infarction model, Zhao et al showed that human UC-MSC-EVs reduced cardiomyocyte apoptosis, promoted endothelial proliferation and angiogenesis, reduced cardiac fibrosis and improved systolic cardiac function.[62] Likewise in a rat myocardial infarction model, Bian and colleagues demonstrated that BM-MSC-EVs promoted angiogenesis in the ischemic heart.[63]

Subsequent studies showed that miR-210 mediates angiogenic effects of MSC-EVs via its target gene Efna-3.[65] miR-210 silencing abolished angiogenesis in an acute mouse myocardial infarct model,[65] which suggested that miR-210 is essential for MSC-EV-induced actions on the microvascular endothelium.[66] In another study in a mouse myocardial infarction model, miR-125b mediated effects of MSC-EVs on myocardial injury and cardiomyocyte survival via mechanisms involving proapoptotic gene p53 and BAK1 expression in cardiomyocytes.[67] miR-125b knockdown reversed the cardioprotective effect of MSC-EVs on myocardial injury, cardiomyocyte survival and p53 and BAK1 expression,[67] displaying that miR-125b was indispensable for the actions of MSC-EVs in myocardial infarction. Again in a mouse model of myocardial infarction, miR-125b achieved cardioprotective effects by reducing autophagic flux in infarcted hearts.[68] This effect was abolished after anti-miR-125b oligonucleotide delivery.[68] miR-125b-5p regulates cell survival by targeting BAK1 (brassinosteroid insensitive 1-associated receptor kinase 1) and TRAF6 (tumor necrosis factor receptor-associated factor 6) and promotes cardiomyocyte proliferation.[67,69,70] Liang et al showed that miR-125a is highly enriched in AD-MSC-EVs.[71] The latter are taken up by endothelial cells, promoting angiogenesis by downregulating DLL4,[71] a key regulator of lateral inhibition during angiogenesis.[72] As a consequence, an increased number of endothelial tip cells were observed.[71] AD-MSC-EV-derived miR-93-5p was shown to reduce myocardial injury in vitro and in vivo via mechanisms involving reduction of hypoxia-induced autophagy and inflammatory cytokine expression by targeting Atg7 and TLR4.[73]

EV proteins also play pivotal roles in myocardial ischemia. Using a mass spectrometry technique, Anderson and colleagues identified 1927 proteins in MSC-EVs, among which were several proteins with robust angiogenic signaling function.[74] Interestingly, the EV proteome was found to depend on the extracellular micromilieu of their host cells. As such, the authors mentioned above found higher levels of angiogenesis-related proteins in EVs derived from MSCs obtained under conditions of peripheral arterial disease compared with standard conditions. These belong to the PDGF (platelet derived growth factor), epidermal growth factor, fibroblast growth factor, and most notably NF-kB (nuclear factor-kB) signaling pathways. By functional in vitro validation using a specific inhibitor, NF-kB signaling was identified as a key mediator of MSC-EV-induced angiogenesis in endothelial cells.[74] Zhang et al demonstrated that MSC-EVs promote proliferation and migration of endothelial cells by activating the Wnt/β-catenin pathway, and Wnt4 (Wnt Family Member 4) in

TABLE 1 Targets and functions of MSC-EV-derived miRNAs and proteins in cerebral ischemia

EV content	Target	Function	Reference
miR-25	BNIP3	Autophagy regulation	45
miR-133b	CTGF	Decreases glial scar formation	46,47
	RhoA	Increases regeneration of the corticospinal tract	48,49
miR-17-92	PTEN/mTOR	Proaxonogenesis effect	52
miR-138-5p	LCN2	Promotes proliferation and inhibit apoptosis of astrocytes	53
miR-let-7b	TLR4/NF-κB/STAT3/PKB	Induces macrophage polarization and inflammatory ablation	54
miR-134	Caspase-8	Prevents oligodendrocyte apoptosis	55
miR-26a	Histone deacetylase 6	Induces neurogenesis	56
Protein clusters	STAT3	Inhibits autophagy and promote angiogenesis	60

TABLE 2 Targets and functions of MSC-EV-derived miRNAs and proteins in myocardial infarction

EV content	Target	Function	Reference
miR-210	Ctgf/HGF/Ptp1b/VEGF	Induces angiogenesis	65,66
miR-125b	Autophagic flux	Cardioprotective effect	67,68
miR-125b-5p	p53 and BAK1	Cardioprotective effect	69,70
miR-125a	DLL4-Notch	Induces angiogenesis	71,72
miR-93-5p	Atg7 and Toll-like receptor 4	Suppresses autophagy and inflammatory	73
Wnt4	Wnt/b-Catenin	Promotes proliferation and migration of endothelial cells	75
CXCR4	PI3K/Akt-associated signaling pathways	Promotes angiogenesis and reduced infarct size	76
PDGF-D	PDGF-D/PDGFR-b pathway	Promotes angiogenesis	77
Jagged-1	Notch signaling pathway	Improves angiogenesis	78

MSC-EVs was critical for the activation of Wnt/β-catenin signaling.[75] Kang et al indicated that CXCR4 (C-X-C motif chemokine receptor 4) in MSC-EVs suppresses apoptosis by activating PI3K (phosphoinositide 3-kinase)/Akt signaling, which in turn promotes angiogenesis and reduces infarct size in a rat myocardial infarction model.[76] In line with this, PDGF-D in MSC-EVs was found to promote angiogenesis in a rat myocardial infarction model by activating the PDGF receptor-β pathway.[77] Angiogenesis was abrogated in endothelial cells treated with EVs obtained from MSCs transfected with PDGF-D siRNA.[77] In endothelial cells, MSC-EV-derived Jagged-1 was found to induce angiogenesis in a HIF-1α (hypoxia-inducible factor-1α) dependent way by activating Notch signaling.[78] This effect was abolished by anti-Jagged-1 antibody delivery.[78] The targets and functions of MSC-EV-related miRNAs and proteins in myocardial infarction are summarized in Table 2.

5 | COMMON MOLECULAR MECHANISMS AND SIGNALING TARGETS OF EVs IN CEREBRAL AND MYOCARDIAL ISCHEMIA

Ischemic stroke and myocardial infarction share several pathophysiological mechanisms and risk factors. Ischemic stroke has been shown to decrease the left ventricular output, increase cardiac interstitial fibrosis, and induce myocardial hypertrophy after 4 weeks in a mouse model.[79] Cardiac dysfunction or heart failure has conversely been shown to elevate the risk of ischemic stroke.[10] By inducing heart failure, myocardial infarction compromises stroke recovery.[2] EVs are important signals mediating heart-brain interactions, and miRNAs may mediate some of them.[2] In the following, we focus on signaling pathways and molecular mechanisms of EVs that are common to the heart and the brain. Consequently, we will not further elucidate pathways where EVs impact either the heart or the brain only, which will indirectly affect the other organ according to the heart-brain axis (see before).

Several miRNAs are contained in MSC-EVs,[33] such as miR-210, miR-126, and miR-17-92 clusters. miR-210 is highly enriched in MSC-EVs and plays a critical role in angiogenesis of the brain[80] and the heart.[81] Under both pathological conditions, miR-210 regulates apoptosis by modifying the expression of hepatocyte growth factor (HGF), which has previously been shown to amplify angiogenesis, neurogenesis and synaptogenesis.[82] Indeed, miRNA-210 promoted neovascularization and NPC migration in a mouse ischemic stroke model.[83] The latter suggests that miR-210 may induce cerebral and cardiac angiogenesis through the same pathway. In line with this, miR-126 controls angiogenesis and maintains vascular integrity.[84] Stroke significantly decreases both circulating and myocardial miR-126 expression.[84] In miR-126 overexpressing mice, the expression of the

inflammatory factors MCP-1 and VCAM-1 was reduced, which suggested inhibitory effects on heart inflammation and oxidative stress after ischemic stroke.[84] Nevertheless, the expression level of miR-126 did not affect the lesion volume in a mouse ischemic stroke model.[84] Apart from miR-126, the miR-17-92 cluster is also highly enriched in MSC-EVs, and plays an essential role in mediating neural progenitor cell function by increasing cell proliferation and inhibiting cell death.[85] Meanwhile, miR-17-92 has been recognized to be also critical for cardiomyocyte proliferation in embryonic and postnatal hearts.[86] One may therefore suggest that miR-17-92 clusters are essential for the ontogenesis of both the heart and the brain. Nevertheless, the miR-17-92 cluster also exerts direct protective effects in the ischemic heart and brain by targeting the PI3K/AKT and the MAPK/ERK pathways.[87,88]

Oxidative stress is one of the common pathophysiological factors in ischemic stroke and myocardial infarction.[89] Intravesicular miR-30d-5p reverses acute ischemic stroke injury by promoting M2 microglia/macrophage polarization.[90] miR-30d-5p is a critical regulator of apoptotic injury after myocardial infarction in mice.[91] miR-30d-5p was suggested to be used as a potential target for early diagnosis in acute myocardial infarction.[92] Other EV-associated miRNAs such as miR-152-3p and let-7i-5p are deeply involved in hypoxia-induced cell death of cardiomyocytes in vitro (H9c2 cells) by targeting Atg12 and Faslg.[93] miR-152-3p also protects neurons from oxygen-glucose-deprivation injury by regulating the Nrf2/ARE pathway.[94] Furthermore, miR-124 induces neuroprotection in a model of ischemic stroke in mice and improves axonal growth by targeting the PI3K/AKT/mTOR pathway,[95] whereas decreased miR-124 expression can reduce cardiomyocyte apoptosis in a mouse model of myocardial infarction.[96] The different functions of miR-124 in the heart and the brain suggest that the same miRNA may affect different pathways in the two organs. Dissociating observations, on the other hand, were described for miR-122. Upregulation of the latter was found to protect against neuronal death in an ischemic stroke model in mice by targeting FOXO3,[97] while the same miRNA inhibited GATA-4 and increased apoptotic injury in a rat myocardial infarct model.[98] Apparently, different signaling cascades are affected by miR-122 that have opposite effects on cell survival. As such, systemic delivery of EVs containing miR-122 might affect the ischemic brain and heart in conflicting ways, yielding unpredictable results.

It has been shown that MSC-EVs and EVs from other cell sources affect peripheral immunity in myocardial infarction,[99] an observation also made after ischemic stroke in mice.[39,100] MSC-EVs affect several immune cell subsets, such as T and B lymphocytes. AD-MSC-EVs can depress T cell activity by transferring cytokines such as IL-4 or IL-10.[101] Furthermore, MSC-EVs express and secrete PD-1 ligands to suppress T cell activity.[102] T cells and B cells have complex roles in ischemic stroke.[103] Regulatory B cells were shown to protect against acute ischemic damage.[104-106] MSC-EVs inhibit both B cell proliferation and differentiation in a dose-dependent fashion.[107] Since B cell activation promotes the production of anti-CNS autoantibodies after stroke,[103] one might suggest that MSC-EVs can achieve cytoprotection by modulating B cell activation in both ischemic stroke and myocardial infarction. In our previous study, we observed a peripheral immunomodulation due to MSC-EVs[39] and NPC-EVs.[28] The modulation of peripheral immunity by stem-cell-derived EVs holds great therapeutic potential, albeit plenty of questions remain unanswered. Targets and functions of the same EV miRNAs in myocardial infarction and cerebral ischemia are summarized in Table 3.

6 | NEW PERSPECTIVES IN EV-BASED THERAPY

Although EVs pass the blood-brain barrier and the cell membrane,[108] the number of EV particles that reaches target tissues is limited. Bioengineering approaches might help to increase tissue EV uptake. Among different strategies, enhancing the targeting properties of EVs by means of well-defined homing peptides is a particularly promising approach.[109] Zhu et al used DBCO-sulfo-NHS (dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester) as a linker and added IMT (ischemic myocardium-targeted) peptide as a ligand to modify the surface of MSC-EVs, which significantly increased the uptake of EVs into the myocardium.[67] Vandergriff et al used a cardiac homing peptide conjugated with cardiosphere-derived EVs to increase the specific targeting ability of the myocardium.[110] The group used DOPE-NHS (dioleoylphosphatidylethanolamine N-hydroxysuccinimide) to conjunct EVs and peptides, which increased EV uptake in cardiomyocytes in vitro and increased functional recovery in a rat myocardial infarction model in vivo.[110] Wang et al synthesized an ischemic myocardium-targeting peptide and formed a conjunction with it on an engineered EV-enriched membrane protein (Lamp2b).[111] Such engineered EVs increased the targeting ability to the myocardium and decreased the trapped EV numbers in nontarget organs. Compared with native EVs, engineered EVs significantly inhibited the inflammatory response of the myocardium, increased angiogenesis and reduced apoptotic cell injury.[111]

Other approaches imply pH-sensitive fusogenic peptides and cationic lipids to improve the cytosolic release of EV (ie, exosomal) contents such as genes and proteins.[112] GALA (Glu-Ala-Leu-Ala), a pH-sensitive fusogenic peptide, was shown to improve the fusion of EV and endosome.[112] However, defining the optimal concentration of GALA is critical for this approach. High concentrations of GALA, for instance, did not improve the fusion of EVs and cells but decreased the number of EVs taken up by target cells.[112] Kooijmans et al developed a strategy by conjugating EVs and nanobodies with PEG to enhance the half-life time of EVs and prolonge exposure to their target-specific receptor.[113] Such PEGylated EVs lasted over 60 minutes in the bloodstream, while regular EVs persisted only 10 minutes. PEGylation protected EVs from interacting with the harsh environment of the blood, which prolonged the half-life time of EVs.[113] Although PEGylation decreased the mutual EV-cell interaction, it improved the stability and targetability of EV-nanobody complexes.[113,114]

The application of EVs does not only offer therapeutic chances for either disease. EV application implies potential pitfalls, among which is the low production rate of these vesicles. Besides, controlling the natural EV cargo imposes challenges since source cells respond differently at various cell stages or changes in the

TABLE 3 Common targets/functions of EV-derived miRNAs and proteins from Tables 1 and 2 in myocardial infarction and cerebral ischemia

	Target		Function		
EV content	Brain	Heart	Brain	Heart	Reference
miR-210	Hepatocyte growth factor	Hepatocyte growth factor	Promotes neovascularization and NPC migration	Angiogenesis	81-83
miR-126	MCP-1 VCAM-1	MCP-1 VCAM-1	Angiogenesis	Anti-inflammation and oxidative stress	84
miR-17-92 cluster	PI3K/AKT MAPK/ERK	PI3K/AKT MAPK/ERK	Protective effects	Protective effects	85-88
miR-30d-5p	Autophagy related pathway	Picalm and Skil	Reverses acute ischemic stroke injury	Reduce apoptosis in cardiomyocytes	90-92
miR-152-3p	Nrf2/ARE	Atg12 and Faslg	Reduces oxygen–glucose-deprivation/ reoxygenation-induced injury	Reduce apoptosis in H9c2 cells	93,94
miR-124	PI3K/AKT/mTOR	STAT3	Neuroprotective effects	Increase cardiomyocyte apoptosis	95,96
miR-122	FOXO3	GATA-4	Neuroprotective effects	Increase cardiomyocyte apoptosis	97,98
IL-4, IL-10	T lymphocytes	T lymphocytes	Inhibits the activity of T cells	Depress the activity of T cells	101
PD-1 ligands	T lymphocytes	T lymphocytes	Inhibits the activity of T cells	Depress the activity of T cells	102

microenvironment.[115] Synthetic EVs mimicking the characteristics of endogenous EVs may therefore be possible alternatives. EV mimics allow for custom cargo selection and a scalable well-characterized drug delivery system. Jang et al synthesized doxorubicin-loaded EV-mimetic nanovesicles by repeating the extrusion of human U937 monocytes through polycarbonate membranes and incubating EV-mimetic nanovesicles with doxorubicin after the extraction process.[116] The systemic administration of these doxorubicin-loaded EV-mimetic nanovesicles significantly improved the targeting ability and delivery of drugs to the tumor site of CT26 mice. Oh et al produced EV-mimetic nanovesicles from a murine pancreatic β-cell line to investigate their therapeutic potential in a diabetic immunocompromised mouse model using the same method as Jang et al,[117] which improved the differentiation of insulin-producing cells and maintenance of physiological glucose levels in vivo. Sato and colleagues developed a two-step protocol for producing hybrid EV-liposome constructs instead of serial extrusion.[118] They created hybrids of a PEG conjugated liposome and EVs using the freeze-thaw method. This procedure avoided the contamination of conjugated material. The engineered hybrid liposome-EVs increased the cellular uptake by a factor of 2 compared with unmodified EVs. Bioengineering techniques are expected to promote the field in the nearer future.

7 | CONCLUSION AND FUTURE PERSPECTIVES

EVs undoubtedly have great potential as a therapeutic tool in ischemic stroke and myocardial infarction. Since EVs play a critical role in intracellular communication, they are essential to numerous biological functions such as tissue regeneration, immune response regulation, and tissue remodeling. Many studies indicated that EVs can inherit the advantages of stem cell transplantation and avoid the risks of cell transplantation at the same time. Furthermore, due to their low immunogenicity, EVs can be modified into an ideal therapeutic delivery vehicle for stroke patients because of their significant potential for postischemic tissue regeneration. Both miRNAs and proteins located in EVs can induce acute cellular protection and stimulate postischemic tissue regeneration by affecting various signaling pathways. However, the same EV miRNA or protein may affect different signaling pathways in the heart or the brain, with opposite effects on disease outcomes. More research on the mechanisms of EV-miRNAs is needed to appropriately judge the side effects of EV treatment. Future research should intensify focus on the EV impact on the heart-brain axis, especially on the mutual interactions of these two organs under ischemic conditions. Tissue engineering approaches have provided significant progress, making EVs attractive nanocarriers for nucleic acids, proteins, small molecules or even drugs to reduce EV-based side effects and increase the targeting ability of EVs. Solid preclinical data will be required for such strategies, before clinical application is in order.

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CONFLICT OF INTEREST

D.H. declared U.S. patent on exosomes for clinical use in stroke and myocardial infarct patients. The other authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

X.Z., T.R.D.: designed and wrote the article, approved the final version of the paper; D.M.H., M.B.: wrote the manuscript, approved the final version of the paper.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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