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Adipose-derived stem cell extracellular vesicles: A systematic review☆

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Summary *Introduction:* Extracellular vesicles (EVs) are cell-secreted packages that deliver cargo to target cells to effect functional and phenotypic changes. They are secreted by many different cell types, including adipose-derived stem cells (ADSCs), which are a promising field of study in regenerative medicine. Our aim was to perform a systematic review of the literature to summarize the scientific work that has been conducted on ADSC EVs to date.
Methods: The Pubmed database was queried with keywords (and variations of) "adipose derived stem cell," "stromal vascular fraction," and "extracellular vesicles." We excluded review papers, then manually screened articles based on title and abstract. Full-text articles were assessed for eligibility to include in final review.
Results: While an extensive body of research exists on EVs, a much smaller proportion of that is original research on ADSC EVs. Of 44 manuscripts that met our database search criteria, 21 articles were selected for our systematic review.
Conclusion: ADSC EVs were found to exert effects on angiogenesis, cell survival and apoptosis, inflammation, tissue regeneration, and reduction of disease pathology. Further studies examine characteristics of ADSC EVs. Future work should aim to further detail the safety profiles of ADSC

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EVs given their potential for cell-based therapies. The body of research studies characterizing ADSC EVs continues to expand, and much work remains to be done before human pilot studies can be considered. To our knowledge, we offer the first systematic review summarizing the research on ADSC EVs and their determined roles to date.

Introduction

The use of adipose-derived stem cells (ADSCs) in regenerative medicine has sparked the interest of clinical scientists and surgeons alike given the ease of access, abundance, high proliferative rate, and lower senescence compared with other types of stem cells.[1,2] In light of the paradigm shift to a position that mesenchymal stem cells primarily exert their actions through paracrine (cell-to-cell) signaling,[3] investigators have diverted much of their attention to extracellular vesicles (EVs), plasma membrane-derived signalers that mediate their target effects through their contents: microRNA, mRNA, lipids, and proteins.[4-8] The acellular nature of these vesicles, which reduces the potential for tumorigenicity and immunogenicity, as well as the lipid membrane packaging of contents, which increases their stability, are primary reasons that many are excited for EVs to one day serve as an off-the-shelf therapeutic which can be used for the treatment of a host of diseases and in various regenerative medicine applications.[9]

Because various studies use different criteria to classify cell-secreted particles, it has been recommended that authors use "extracellular vesicles" as a broad generic term.[10-12] Generally, most classifications include microvesicles (50–1000 nm in size) and exosomes (30–100 nm) as the two main subsets of secreted vesicles.[7,10-14] To date, there is no gold standard for discrimination between particular subsets of EVs.[7,11,15] As such, we will use the general term "EVs" to describe all these particles.

Extracellular vesicles are produced by a variety of cell types, including lymphocytes, dendritic cells, platelets, neurons, epithelial cells, and adipocytes.[16] They can be formed one of two ways: either by direct outward budding of the cell membrane, or by originating from endosomes, the cellular compartments that store, recycle, or designate their contents for degradation. Inward budding of the endosome generates intraluminal vesicles. When an endosome has accumulated a multitude of these vesicles, this multivesicular body (MVB) has one of two fates: fusion with a lysosome, resulting in degradation of its contents, or fusion with the plasma membrane, releasing the EV into the extracellular space.[10] Once an EV is released via endosome or direct budding, it can then reach its target cell, fuse with its membrane, and deliver its cargo to effect functional or phenotypic changes (Figure 1).

The cargo of EVs is highly dependent upon the cell of origin and mechanism of generation.[17] They can contain genetic material such as mRNA, miRNA or noncoding RNA, as well as proteins, growth factors, and cytokines.[7,14] Specific biomarkers in the membranes of EVs attract them to particular cells, and a single cell can produce a heterogeneous population of EVs with differing markers and compositions.[18,19] By delivering their cargo, EVs are able to effect cellular changes, such as endothelial cell EVs promoting angiogenesis through delivery of growth factors.[20]

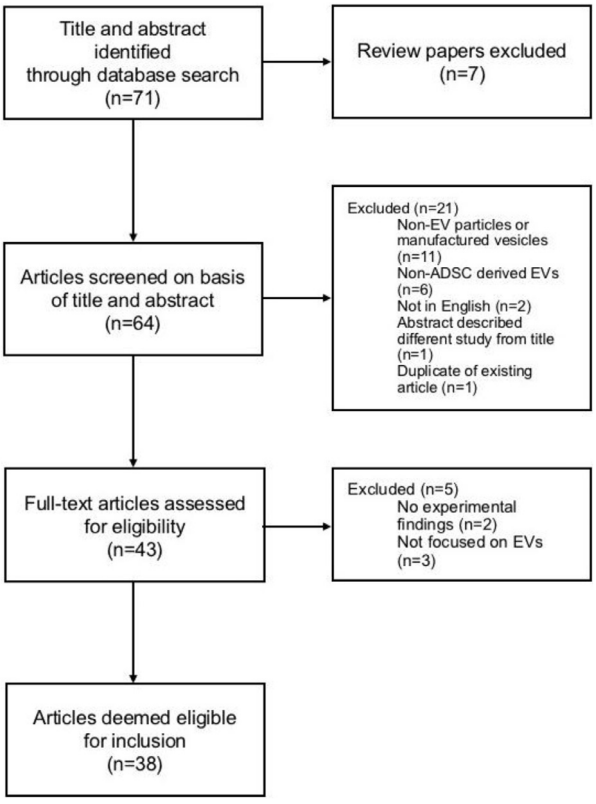

Figure 1 Flowchart of review design.

While EVs from different sources have been shown to share similar contents, they have also been found to contain molecules from their parent cells, and even carry out similar functions as their originators. For example, one of the first immunologic studies on EVs found that B-lymphocytes produced antigen-presenting EVs that induced a specific T-cell response.[21] RNA and proteins extracted from the EVs from glioblastoma cells have been shown to have tumor-enhancing properties.[22]

EVs have become a topic of immense scientific interest as researchers endeavor to identify small molecules, both synthetic and naturally occurring, that can serve as the next generation of therapeutics. Their potential is made even more attractive by the fact that they are biocompatible, generate a minimal immune response, and can be harvested from patients,[23] paving the way for a new approach to precision medicine. The therapeutic power of EVs derived from mesenchymal stem cells (MSC) was first demonstrated in 2009 by Bruno et al., in a study which found that EVs triggered healing of injured kidney tubular cells through mRNA

Table 1 Search terms.

Database	PubMed
Date	03/30/18
Terms	((((((ADSC[Title/Abstract] OR hADSC[Title/Abstract] OR "Adipose derived stem cell"[Title/Abstract] OR "Adipose derived stem cells"[Title/Abstract] OR "Adipose-derived stem cell"[Title/Abstract] OR "Adipose-derived stem cells"[Title/Abstract] OR "Adipose stromal cell"[Title/Abstract] OR "Adipose stromal cells"[Title/Abstract] OR "Adipose-stromal cell"[Title/Abstract] OR "Adipose-stromal cells"[Title/Abstract] OR "Adipose derived stromal cell"[Title/Abstract] OR "Adipose derived stromal cells"[Title/Abstract] OR "Adipose-derived stromal cell"[Title/Abstract] OR "Adipose-derived stromal cells"[Title/Abstract] OR "SVF"[Title/Abstract] OR "stromal vascular fraction"[Title/Abstract] OR "stromal-vascular-fraction"[Title/Abstract] OR "stromal-vascular-fractions"[Title/Abstract] OR "stromal-vascular-fraction"[Title/Abstract]) OR lipoaspirate)))))) AND ((extracellular vesicles OR exosomes OR apoptotic bodies))
Date	03/30/18, 02/07/19
Terms	((((((ADSC[Title/Abstract] OR hADSC[Title/Abstract] OR "Adipose derived stem cell"[Title/Abstract] OR "Adipose derived stem cells"[Title/Abstract] OR "Adipose-derived stem cell"[Title/Abstract] OR "Adipose-derived stem cells"[Title/Abstract] OR "Adipose stromal cell"[Title/Abstract] OR "Adipose stromal cells"[Title/Abstract] OR "Adipose-stromal cell"[Title/Abstract] OR "Adipose-stromal cells"[Title/Abstract] OR "Adipose derived stromal cell"[Title/Abstract] OR "Adipose derived stromal cells"[Title/Abstract] OR "Adipose-derived stromal cell"[Title/Abstract] OR "Adipose-derived stromal cells"[Title/Abstract] OR "SVF"[Title/Abstract] OR "stromal vascular fraction"[Title/Abstract] OR "stromal-vascular-fraction"[Title/Abstract] OR "stromal-vascular-fractions"[Title/Abstract] OR "stromal-vascular-fraction"[Title/Abstract]) OR lipoaspirate)))))) AND ((extracellular vesicles OR exosomes OR apoptotic bodies OR microparticles))

delivery. Since then, numerous studies have found that MSC EVs have therapeutic potential in kidney injury, myocardial ischemia, and stroke. Because of their greater accessibility, ADSCs which are a subtype of MSCs, are being increasingly explored as a source of EVs; therefore, we sought to evaluate the current applications of ADSC EVs.

Key objectives

Our primary goal is to provide an overview of original studies to date performed on ADSC-derived EVs by reviewing all published data. Secondarily, we aim to determine gaps in the current literature in order to identify future avenues for research.

Methods

Search strategy

We performed a systematic search in the PubMed database on February 6, 2019 for studies involving EVs derived from ADSCs as of December 31, 2018. A search strategy was generated using the following terms: "adipose-derived stem cells," "stromal vascular fraction," "exosomes," "extracellular vesicles," and synonyms (Table 1). Retrieved articles were stored in a database and duplicates were removed.

Study selection

Two authors reviewed both the abstracts and titles (D.E.W. and P.J.F.S.). Reviews were excluded, and only original studies were used. Of the initial selection, the two authors reviewed full texts independently and included them if they met inclusion criteria (as shown in Table 2). If article eligibility was disputed, the two authors along with a third (D.B.) discussed the article and, based on consensus, included or excluded it. Information from each trial was extracted by two authors independently (D.E.W. and P.J.F.S.) and organized into clinical context, ADSC origin, EV isolation technique, experimental data, and statistical significance.

Results

Of the 71 references initially obtained, 38 studies of ADSC EVs were included in our systematic review (Figure 2). The studies were organized based on the clinical role and context in which the ADSC EVs were studied. If a study identifies multiple roles for ADSC EVs, each finding is discussed separately in its corresponding section.

The majority of researchers employed ultracentrifugation for the isolation and purification of exosomes. This is the assumed method of harvesting in each study unless otherwise specified. It is noteworthy to point out that a much larger body of research exists on mesenchymal stem cell-derived EVs. However, this systematic review is designed to summarize the literature particularly as it pertains to ADSC EVs.

Angiogenesis

Of the studies that identified angiogenic roles of ADSC EVs, four utilized human umbilical vein endothelial cells (HUVECs) to verify this function.[24-26] Kang et al. measured HUVEC migration with the Boyden chamber technique. Human ADSCs were preconditioned (MV-P) or not (MV) with endothelial cell medium containing growth factors. RNA

Table 2 Summary of included studies.

Study Context	Author	Source of ADSC	Effect of ADSC EVs
Neurological disorders			
Alzheimer's disease	Katsuda et al., 2015	Human	Have Aβ-degrading capacity
Alzheimer's disease	Katsuda et al., 2013	Human	Deliver enzymatically active neprilysin which results in decreased Aβ amyloid production
Alzheimer's disease	Lee et al., 2018	Human	Reduce β-amyloid pathology and apoptosis
Amyotrophic lateral sclerosis	Bonafede et al., 2016	Murine	Protective against oxidative stress
Amyotrophic lateral sclerosis	Lee M et al., 2016	Human	Modulate cellular phenotypes of ALS including SOD-1 aggregation and mitochondrial dysfunction
Huntington's disease	Lee M et al., 2016	Human	Modulate representative cellular phenotypes of HD
Brain injury	El Bassit et al., 2017	Human	Promote survival of mouse hippocampal neurons
Traumatic brain injury	Patel et al., 2019	Human	Motor function recovery and reduction in cortical brain injury
Acute ischemic stroke	Jiang et al., 2018	Rat	Decrease cerebral area of infarction by suppressing autophagy and promoting microglia/macrophage polarization
Organ-specific disorders			
Corneal stromal fibroblast viability	Shen et al., 2018	New Zealand white rabbits	Promote CSC viability regulation and ECM remodeling
Emphysema	Kim et al., 2017	Human	Nanovesicles demonstrate proliferative capacity similar to ADSCs in a murine emphysema model through FGF2 signaling
Liver fibrosis	Qu et al., 2017	Mice	Selectively transfer miR-181-5p to damaged liver cells
Acute liver failure	Jin et al., 2018	Rats	Increase 72-hour survival rate by $>$70% when injected into bloodstream
Myocardial ischemic injuries	Luo et al., 2017	Rats	Prevent myocardial damage by protecting myocardial cells from apoptosis, inflammation, fibrosis, and increased angiogenesis
Myocardial ischemic injuries	Pan et al., 2019	Rats	Reduce myocardial inflammation and area of reactive fibrosis through decreasing inflammatory and fibrotic factors
Myocardial ischemic injuries	Liu et al., 2018	Rats	Protect myocardium from hypoxia-induced autophagy and inflammatory cytokine expression via miR-93-5p
Genitourinary			
Erectile dysfunction	Chen et al., 2017	Human	Improvement in erectile dysfunction in a diabetic rat model
Erectile dysfunction	Zhu et al., 2018	Rats	Transport key functional miRNAs to target cells in a specific manner to improve functional recovery or to activate endogenous repair mechanisms
Erectile dysfunction	Li et al., 2018	Commercially available hADSCs	Increased mean intracavernous pressure/mean arterial pressure ratio after cavernous nerve injury
Prostate cancer (PCa)	Takahara et al., 2016	Commercially available hADSCs	Inhibit PCa growth, inducing PCa cell apoptosis with reduced activity of BclxL, at least in part, by miR-145
Stress urinary incontinence	Ni et al., 2018	Human	Promote functional and histological recovery from induced stress urinary incontinence
Endocrine			
Obesity and metabolic disorders	Zhao et al., 2018	Mouse	Facilitate immune and metabolic homeostasis in white adipose tissue
Plastic Surgery & Regenerative Medicine			
Fat grafting	Han et al., 2018	Human	Promote survival, neovascularization, and reduce inflammation in fat grafting
Human dermal fibroblast migration	Choi et al., 2017	Human	Prosurvival in human dermal fibroblast migration *ex vivo*
Human dermal fibroblast migration	Cooper et al., 2018	Human	Increased rate of human dermal fibroblast migration and wound closure
Human dermal fibroblast migration	Zhang et al., 2018	Human	Increased rate of human dermal fibroblast migration and reduction in wound area
Angiogenesis	Kang et al., 2016	Human	Encourage angiogenesis of human umbilical vein endothelial cells
Angiogenesis	Yang et al., 2018	Rats	Promote angiogenesis of brain microvascular endothelial cells
Bone regeneration with cell-free tissue-engineered bone	Li et al., 2018	Commercially available hADSCs & hBMSCs	Novel cell-free system comprised of hADSC-derived exosomes and PLGA/pDA scaffold provides new therapeutic potential for bone tissue engineering

(*continued on next page*)

Table 2 (*continued*)

Study Context	Author	Source of ADSC	Effect of ADSC EVs
Skin flap survival in plastic surgery	Pu et al., 2017	Human	Improvement in skin recovery through IL-6 release
Skin flap survival in plastic surgery	Bai et al., 2018	Human	Increased flap survival, improved outcomes compared to normal EVs
Tissue regeneration in periodontitis	Mohammed et al., 2018	Rats	Increase in area of newly formed periodontal tissue, added proliferation and organization
Peripheral nerve regeneration	Ching et al., 2018	Rats	Enhancement of murine neurite outgrowth *in vitro*
Lymphangiogenesis	Wang et al., 2018	Human	Increased lymphatic endothelial cell proliferation, migration, and tube formation via miR-132
Other			
Electroporation for drug delivery	Johnsen et al., 2016	Human	Electroporation does not change the stimulatory capacity of exosomes on glioblastoma multiforme cells. It does, however, cause aggregation of the exosomes, which is attenuated by trehalose medium
Safety of oncogenic ADSC exosomes	Garcia-Contreras et al., 2014	Human	ADSC derived exosomes from urological neoplastic patients are phenotypically equivalent for therapeutic purposes.

extraction and analysis of EV contents showed that HUVEC migration (control: 45.3±12.8 cells; MV: 127.0±14.3 cells; $p<0.01$) and tube formation (control: 1.00±0.10; MV: 1.67±0.15; $p<0.01$) were induced by EVs. RNA analysis identified miR-31 as the only proangiogenic RNA, and its silencing reduced angiogenic growth from MV-P in an ex vivo aortic ring assay ($p<0.01$).

Han et al. examined the effects of human ADSC EVs produced under normoxic and hypoxic conditions on HUVEC migration (via a wound healing assay) and capillary network formation (by measuring tube length). They found that hypoxic ADSC EVs significantly increased migration and capillary network formation (all $p<0.05$) compared to normoxia.[25]

Similarly, Bai et al. found that human ADSC EVs improved proliferative capacity, migration, and tube formation of HUVECs compared to control ($p<0.05$).[27] They also devised an in vivo model of murine skin flaps subjected to ischemia-reperfusion injury and investigated the effects of ADSC EVs on flap survival, discovering a significantly increased survival ratio when injected with ADSC EVs compared to control ($p<0.05$).

Another study described an in vitro diabetic rat model for erectile dysfunction and found that high-glucose conditions impaired HUVEC tube formation, and rat ADSC EVs stimulated significant, concentration-dependent, pro-angiogenic activity.[26] They also observed increases in intracavernous pressure to mean arterial pressure ratio (ICP/MAP), collagen deposition, and endothelial content. Proangiogenic microRNAs were detected in the cellular and exosome lysates.

Analogously to HUVECs, brain microvascular endothelial cells (BMECs) subjected to oxygen-glucose deprivation then treated with rat ADSC EVs were found to have increased migration distance and angiogenesis, measured by tube length, compared to no EVs ($p<0.05$).[28] These findings suggested possible efficacy of ADSC EVs in acute stroke.

Two additional studies demonstrated proangiogenic potential. Human ADSC EV injection increased survival of extended pectoral flaps on mice compared to control. It was found to be mediated by IL-6, a proangiogenic cytokine that is concentrated in ADSC EVs.[29] An in vitro model of myocardial injury showed that ADSC EVs accelerated tube-like structure formation and migration of rat endothelial progenitor cells after culturing with EVs from rat ADSCs or miR-126-overexpressing ADSCs under hypoxic conditions.[30] Of note, miR-126 was previously shown to attenuate cell damage after myocardial infarction.[31,32] Von Willebrand factor (vWF) assay showed increased angiogenesis in the EV group compared to control ($p<0.01$), and the best therapeutic effect was found in the miR-126 EV treated group ($p<0.001$). Thus, ADSC EVs encourage angiogenesis in several settings, and when subjected to hypoxic conditions, exert a heightened proangiogenic effect.

ADSC EVs have also been found to have positive effects on lymphangiogenesis. Wang and colleagues investigated the role of human ADSC EVs in lymph tissue growth via vascular endothelial growth factor-C (VEGF-C), finding that ADSC EVs co-cultured with recombinant VEGF-C promoted lymphatic endothelial cell proliferation, migration, and tube formation more than the EVs cultured without VEGF-C; miRNA analyses determined this effect to be due to increased concentrations of miR-132 ($p<0.05$).

Prosurvival/anti-apoptosis

Seven studies identified an anti-apoptotic role for ADSC EVs. Chen et al. found that human ADSC EVs restored erectile function in a diabetic murine model.[33] The rats received intracavernous injection of either ADSC-derived EVs, ADSCs, or phosphate buffered saline (PBS). Four weeks post-treatment with EVs or ADSCs showed that ICP/MAP was increased compared to the PBS group ($p<0.05$). Furthermore, Western blot showed increased expression of endothelial markers compared to PBS ($p<0.05$) demonstrating attenuation of apoptosis. Western blot also revealed that protein caspase-3, a protein associated with apoptosis, was increased in the PBS group compared to rats undergoing sham

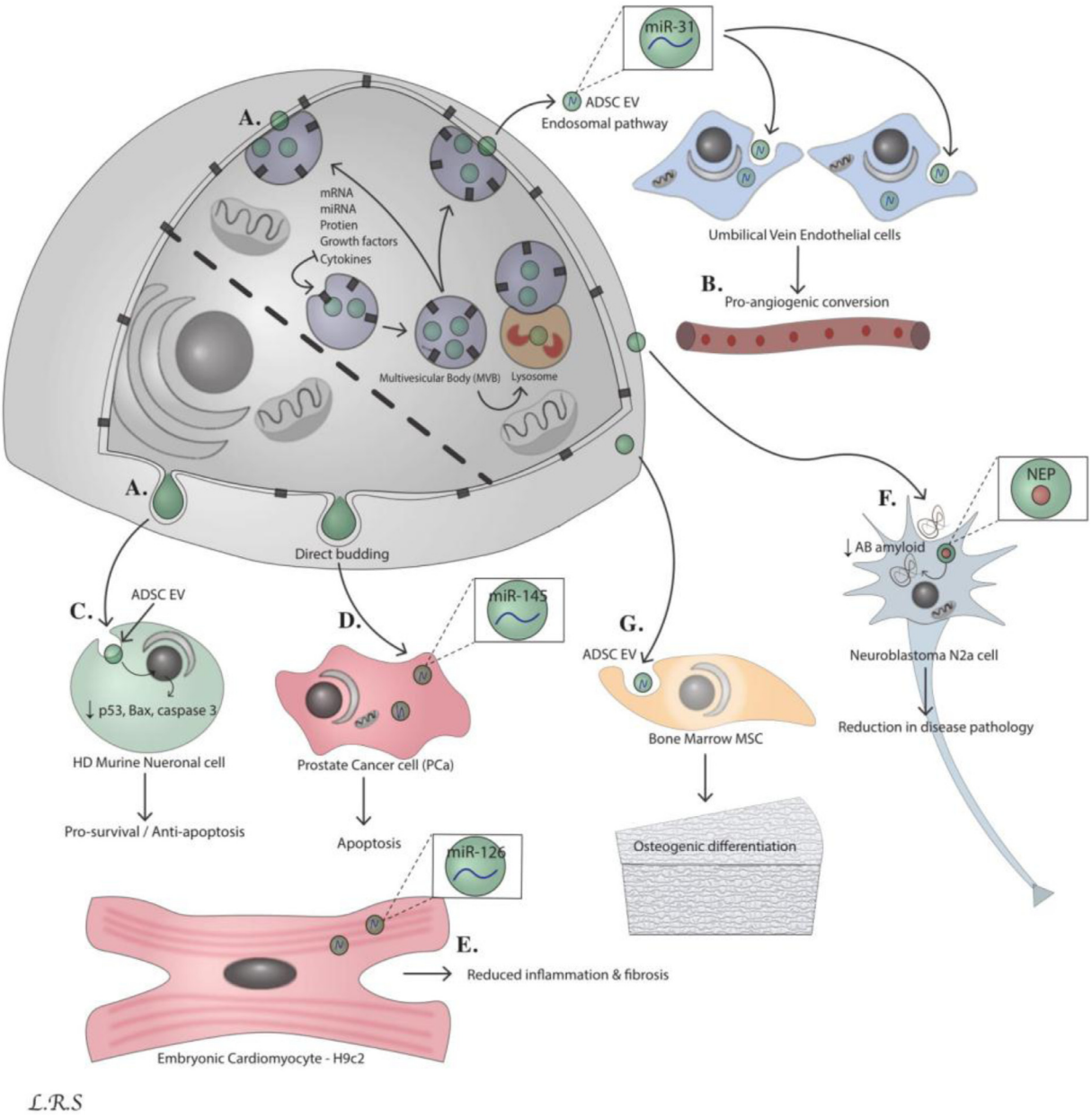

Figure 2 Biogenesis of extracellular vesicles and roles defined in this study. Extracellular vesicles (EVs) are membrane-packed vesicles that deliver cargo to target cells in a process known as paracrine, or cell-to-cell, communication. They are secreted by numerous different cell types, and though their contents can vary widely, they commonly consist of proteins, mRNAs, miRNAs, and noncoding RNAs. They can be purified and are immunologically inert, which makes them attractive as a possible therapeutic option. (A) EVs are formed either by direct budding off the plasma membrane (these are often known as microvesicles), or as vesicles within endosome-derived multivesicular bodies (MVBs), which fuse with the cell surface to release them into the extracellular milieu (these are frequently called exosomes). ADSCs a popular subject of study because of their potential in regenerative medicine and their ease of access; as EVs often contain similar contents as their parent cells and can even fulfill similar roles, they are of significant interest. (B) ADSC EVs have been found to bear proangiogenic capabilities. This effect becomes marked when they are cultured in hypoxic conditions. RNA analysis has identified the proangiogenic miR-31 as responsible for the effect on angiogenesis. (C) Other studies have determined that EVs can also counteract apoptosis or exert a prosurvival effect on cells. (D) One study found that EVs actually increased apoptosis, accelerating the death of cancer cells. (E) EVs evidently have anti-inflammatory abilities as well, as experiments on ADSC EVs. (F) Through effecting changes in diseased protein levels, EVs have also been shown to ameliorate the disease phenotypes of Alzheimer disease, amyotrophic lateral sclerosis, and Huntington disease. (G) EVs have implications in regenerative medicine as well, as they have been demonstrated to encourage osteogenic differentiation and exert proliferative capacity on epithelial cells.

operation, but was decreased with the addition of ADSCs or EVs ($p<0.05$).

In a study by Lee et al., human ADSC EVs reduced apoptosis in R6/2 murine neuronal cells, which express exon 1 of the Huntington disease (HD) gene with 150 CAG repeats.[34] Additionally, apoptosis-associated proteins p53, Bax, and cleaved caspase-3 levels were lower as indicated by Western blot in the groups treated with ADSC EVs compared to the HD control groups ($p<0.05$). In a separate study, the same group demonstrated in an in vitro Alzheimer's disease model that human ADSC EV treatment had similar anti-apoptotic effects on protein concentrations in murine neuronal cells.[35] Western blot showed that anti-apoptotic proteins p53, p73, and caspase-3 were decreased after treatment with ADSC EVs compared to control ($p<0.01$).

Shen et al. demonstrated that rabbit ADSC EVs reduced apoptosis of rabbit corneal stromal cells and promoted their growth.[36] ADSC EV treatment yielded a lower percentage of apoptotic cells via an annexin V apoptosis detection kit ($p=0.01$). Growth measured via deoxyribonucleic acid synthesis found that cell proliferation was positively associated with the concentration of exosomes ($p<0.05$).

According to Bonafede et al., murine ADSC EVs also had an anti-apoptotic effect on amyotrophic lateral sclerosis (ALS) motor neurons subjected to oxidative damage.[37] NSC-34 cells (a murine hybrid of embryonic spinal cord motor neurons with neuroblastoma) transfected in vitro with the ALS gene human superoxide dismutase 1 (hSOD1), followed by exposure to hydrogen peroxide (H_2O_2) with or without EVs, were assessed for cell survival by cell count. Administration of EVs rescued naive cells with a median of 60% cell viability ($p<0.001$) compared to no cell viability with H_2O_2 alone. In all cases, ADSC EVs exerted a protective effect against H_2O_2-induced apoptosis, increasing cell viability in a dose-dependent manner ($p<0.001$).

The Luo study found that rat ADSC EVs exerted an anti-apoptotic effect on injured myocardial cells.[30] They identified apoptotic cells with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL). Both the EV- and miR-126-EV-treated groups had fewer apoptotic cells than the acute myocardial infarction group ($p<0.001$).

The ADSC secretome was also found to increase survival of cells from the HT22 line of mouse hippocampal neuronal precursor cells.[38] The authors had previously found that protein kinase C delta (PKCd) and its splicing variant PKCdII enhanced neuronal survival.[39] Human ADSC EVs increased PKCdII and Bcl2 levels indicated via Western blot compared to no EV treatment and enhanced neuronal survival and proliferation via 5-bromo-2′-deoxyuridine and tetrazolium salt assays (both $p<0.0001$). PCR demonstrated that metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a long noncoding RNA that regulates alternative gene splicing in ADSC EVs, was taken up by HT22 cells ($p<0.0001$). After using antisense oligonucleotides to decrease MALAT1 expression, the resultant EVs did not significantly increase PKCdII splicing ($p<0.0001$).

Two studies identified pro-survival roles for ADSC EVs. Choi et al. demonstrated this in human dermal fibroblasts (HDFs).[40] PCR showed that expression of CD34, collagen type 1, elastin and keratinocyte growth factor increased after treatment with human ADSC EVs ($p<0.05$). EV-induced HDF proliferation, measured by red fluorescent PKH26 in a dose-dependent manner that was markedly greater in HDFs treated at a higher concentration (10 μg/mL) than lower (5 μg/mL; $p<0.05$). Additionally, expression analysis by microarray was conducted for microRNAs that were expressed differentially (>2 fold, $p<0.05$ 95% CI).

Similarly, Han et al. found that human ADSC EVs promote survival, neovascularization, and reduce inflammation in murine fat grafting, and that hypoxia enhances this effect.[25] Human abdominal fat subjected to hypoxia (5% oxygen) or non-hypoxia (20% oxygen) were transplanted into mice. Grafts treated with ADSC EVs in the hypoxic group weighed more at 2 and 8 weeks post-graft than control ($p<0.05$). The hypoxic group also had better outcomes across the board (all $p<0.05$). The control group had more infiltration and fibrosis than the EV groups by hematoxylin and eosin (H&E) staining, and neovascularization was better in EV groups by Doppler (all $p<0.05$). This set of studies demonstrates that administration of ADSC EVs can improve cell survival or reduce cell death.

Apoptosis

In addition to pro-survival and antiapoptotic effects, there is also evidence that EVs impart a pro-apoptotic effect in some instances. Human ADSC EVs induced apoptosis in mice injected with PC3M-luc2 prostate cancer cells.[41] Immunohistochemical studies demonstrated slowed cancer cell progression, significantly increased numbers of apoptotic cells, and reduced activity of antiapoptotic protein B-cell lymphoma extra-large (BclxL) compared to HDFs ($p<0.01$). miR-145 was expressed at levels five-fold higher in ADSCs than HDFs by exosome miRNA array analysis. Subsequent miR-145 knockdown in ADSCs reduced caspase 3/7 and increased BclxL expression. This paper demonstrates a setting in which ADSC EVs can trigger cell death.

Anti-inflammation

The primary finding of the study by Luo et al. was that miR-126-enriched ADSC EVs from rats decreased injury of H9c2 embryonic cardiomyocyte injury in vitro by reducing inflammation factor expression during hypoxia.[30] miR-126 overexpressing ADSCs decreased fibrosis related protein expression from H9c2 cells under hypoxic conditions. In vivo, after treatment of H9c2 cells with miR-126-enriched EVs, inflammatory cytokine and cardiac fibrosis marker expression were decreased. Similarly, Pan et al. found that infarction-induced cardiac inflammation and fibrosis in rats was attenuated by exposure to rat ADSC EVs, as evidenced by reduced levels of TNF-α, IL-6, and IL-1β ($p<0.001$).[42] Correspondingly, staining with a redox indicator revealed decreased area of reactive fibrosis and infarcted tissue in ADSC EV-treated tissue compared to control ($p<0.001$).

In an in vitro mouse hepatic stellate cell model, murine ADSC EVs containing miR-181-5p decreased fibrotic factors fibronectin and collagen I stimulated by transforming growth factor beta (TGF-B).[43] The EVs also reduced fibrosis induced by the potent hepatotoxin carbon tetrachloride.

ADSC EVs from mice also induced significant weight loss and corrected metabolic disorders in high-fat diet (HFD) mice through improved white adipose tissue (WAT) homeostasis and ameliorated WAT inflammation, and remodeling of macrophage phenotypes ($p<0.05$).[44] Macrophages educated by ADSC EVs contributed to ADSC proliferation and expressed high levels of tyrosine hydroxylase, an enzyme involved in catecholamine synthesis, which macrophages mediate in order to promote fat burning.[45]

The Han study also found that human ADSC EVs reduced inflammation in fat grafting.[25] H&E staining demonstrated more lymphocytes and macrophages around the control groups than the groups treated with ADSC EVs at 2 and 4 weeks. At 6 weeks, the control groups showed fibrosis, septae and small cysts. At 8 weeks, the ADSC EV groups had lower levels of necrosis and more evenly distributed adipocytes.

In an in vitro ischemia myocardial damage rat model, Liu et al. showed that rat ADSC EVs exert reduced damage to myocardial tissue.[46] They demonstrated significantly decreased infarct area ($p<0.001$) and suppressed hypoxia-induced autophagy via Western blot quantification of autophagy related proteins Atg7 and Toll-like receptor 4 ($p<0.001$).

In an acute stroke model of mouse rat microglial cells, Jiang et al. demonstrated that rat ADSC EVs decreased the inflammatory cytokine response to ischemia.[47] They also identified that ADSC EVs also induced reversal of microglial conversion to M1 macrophages, which mediate the process of autophagy in brain injury—this was quantified with immunofluorescent staining for M1 marker iNOS (decreased compared to control, $p<0.001$) and microglial marker Iba1 (increased compared to control, $p<0.001$). By quantifying inflammatory factors in most cases, these studies demonstrated the role of ADSC EVs in lessening inflammation.

Reduction in disease pathology

Several studies revealed findings involving ADSC EVs attenuating disease pathology. Katsuda et al. examined neprilysin (NEP), a therapeutic target for Alzheimer Disease (AD) that degrades B-amyloid peptide (AB) in human ADSC EVs.[48] After confirming that ADSC EVs secrete enzymatically active NEP by fluorescence resonance energy transfer, the authors co-cultured ADSCs with neuroblastoma N2a cells and determined by enzyme-linked immunosorbent assay (ELISA) that the ADSC EVs resulted in both decreased intra- and extracellular Aβ levels ($p < 0.001$). In a separate study, Lee et al. also demonstrated a reduction in Aβ levels in murine neuronal stem cells compared to control ($p<0.01$).[35]

Lee et al. demonstrated normalization of disease pathology through attenuation of mitochondrial dysfunction. In an amyotrophic lateral sclerosis (ALS) in vitro murine model with G93A neuronal stem cells, these animals are characterized by increased superoxide dismutase (SOD-1) aggregation. Bovine ADSC EVs reduced SOD-1 aggregation by dot-blot assay and Western blot.[49] The ADSC EVs normalized levels of mitochondrial proteins that were aberrant in the ALS mutants, PGC-1α and p-CREB/CREB ratio ($p<0.01$).

The third study, also led by Lee et al., used a superoxide indicator assay to show that compared to the HD control, human ADSC EVs reduced fluorescence intensity and therefore imparted mitochondrial protection ($p<0.05$).[34] Mutant huntingtin protein (mHtt) aggregations in the nucleus also decreased, confirmed by western blot ($p<0.001$). It is therefore evident that in certain disease models, ADSC EVs have the ability to diminish pathological markers.

Ni et al. induced stress urinary incontinence in rats by pudendal nerve transection or vaginal dilation and then administered injectable human ADSC EVs, displaying that bladder capacity and leak point pressure increased compared to the control group ($p<0.01$).[50] Additionally, increased amounts of striated muscle and peripheral nerve fibers were found on immunofluorescent stain in the ADSC EV group ($p<0.01$).

In an acute liver failure model, Jin et al. introduced rat-derived ADSC EVs into the iliac veins of rats, improving their 72-h survival rate by over 70% compared to controls ($p<0.05$).[51] They also investigated a long coding RNA H19, which is expressed robustly in liver tissues undergoing proliferation, suggesting a role in hepatic regeneration.[52] Silencing of a long coding H19 decreased the hepatocyte survival rate by 40%, demonstrating a potential therapeutic target ($p<0.05$).

Li et al. generated an erectile dysfunction model by bilaterally transecting the cavernous nerves in rats, and found that three weeks following penile injection of human ADSC EVs, the ratio of intracavernous pressure to mean arterial pressure increased compared to no injection ($p<0.05$).[53] Immunofluorescent staining of cavernous tissue revealed attenuated smooth muscle atrophy and increased nerve tissue content in the group treated with ADSC EV injection compared to control ($p<0.05$). These results all demonstrated a reduction in erectile dysfunction pathology mediated by ADSC EVs.

Rats subjected to controlled cortical traumatic brain injury (TBI) were shown to undergo functional and histopathological improvement after treatment with human ADSC EV therapy.[54] Motor assessment demonstrated that EV treatment significantly rescued TBI-associated motor deficits compared to no treatment ($p<0.001$). Therapy with ADSC EVs also significantly reduced the impact and peri-impact areas compared to control ($p<0.01$ and 0.001, respectively).

In their acute stroke model, Jiang et al. demonstrated that rat ADSC EVs suppress the response to acute ischemia.[47] They showed that this occurred through an in vivo rat model, in which the right middle cerebral artery was occluded with suture. Immediately following the ligation procedure, ADSC EVs were injected intravenously into the tail vein, and the animals were subsequently sacrificed for brain sections. The study showed that ADSC EVs decreased the cerebral area of infarction significantly compared to no ADSC EV treatment ($p<0.001$).

Regenerative medicine

Three studies showed that ADSC EVs increased the rate of cell migration of HDFs. In addition to Choi et al., who demonstrated that ADSC EV administration increased the distance of migration of HDFs ($p<0.05$), Cooper and colleagues applied human ADSC EVs to HDFs subjected to mechanical scratch assay by pipette, finding that migration of

HDFs increased by 43% compared to control ($p<0.05$).[40,55] Zhang et al. quantitatively demonstrated faster cutaneous wound healing in rats ($p<0.01$) and that the increased migration of HDFs ($p<0.001$) is due, at least in part, to the phosphatidylinositol 3-kinase (PI3K) and Akt signaling pathway by Western blot analysis of protein levels of Akt and a PI3K inhibitor.[56]

Examining ADSC EV effects on bone growth, Li et al. observed accelerated restoration of critical-sized mouse calvarial defects by combining human ADSC EVs with a poly(lactic-co-glycolic acid) (PLGA) scaffold.[57] ADSC EVs enhanced the proliferation, migration, and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). Immunofluorescence staining showed that PLGA scaffolds coated with polydopamine and EVs recruited more SSEA-4+/CD45− mesenchymal stem cells than PLGA scaffolds coated with polydopamine (PLGA/pDA) in vivo after a week of implantation.

Utilizing a diabetic foot ulcer model in rats, Li et al. found that human ADSC EVs had regenerative effects, decreasing the relative fibrosis rate ($p<0.001$), increasing relative blood vessel density ($p<0.001$), and significantly accelerating cutaneous wound healing measured by decreased ulcerated area ($p<0.001$).[58]

Kim et al. showed that nanovesicles have proliferative capacity similar to ADSCs in a murine emphysema model, mediated through fibroblast growth factor (FGF2) signaling.[59] Nanovesicles were produced by penetrating human ADSCs through polycarbonate filters. The proliferative capacity of artificial nanovesicles was higher than natural EVs on alveolar epithelial MLE12 cells per colorimetric assay CCK8 ($p<0.05$). Mice were intrathecally injected with elastase and then injected with ADSCs, high (1x) and low (1/3×) concentrations of ADSC nanovesicles or ADSC EVs (based on protein amounts). Similar regenerative effects were seen with ADSCs ($p<0.05$) and high and low ADSC nanovesicle concentrations ($p=0.0089$ and 0.0001, respectively), while EVs resulted in no change. FGF2 was found to be elevated in lysates treated with ADSCs or artificial nanovesicles (1/3×) versus the controls ($p<0.05$). These two studies display the regenerative potential of ADSC EVs, in both osteogenesis and epithelial growth.

In a rat model of ligature-induced periodontitis, Mohammed et al. demonstrated that rat-derived ADSC EVs increased the mean area of newly formed tissue ($p<0.05$), occurring at a higher rate than control and a group subjected to scaling and root planing, which is a standard nonsurgical treatment for periodontitis.[60] Histologic examination of the periodontal tissue at four weeks revealed that ADSC EVs induced highly organized tissue compared to control, along with blood vessels and osteoid tissue, which were not present in control.

The regenerative potential of ADSC EVs also extends to nervous tissue. Ching et al. demonstrated that EVs from differentiated Schwann cell-like rat ADSCs significantly increased neurite outgrowth length compared to control in a murine model ($p<0.001$).[61]

Usage and safety

Garcia-Contreras et al. found that ADSC EVs from the subabdominal fat of human urological cancer patients were phenotypically identical to healthy patients.[62] Molecular karyotyping for copy number gains and loss of heterozygosity found no significant difference in population doublings (6.2926±1.394 cancer, 4.8696±1.801 noncancer, $p>0.5$) and no cancer-associated alterations in the cancer group. Both groups of ADSCs demonstrated adipogenic, osteogenic, and chondrogenic differentiation when induced. PCR found similar amounts of miRNA between groups ($p>0.05$).

Johnsen et al. found that electroporation did not change the stimulatory effect of human ADSC EVs on glioblastoma multiforme (GBM) proliferation.[63] Electroporation buffer using trehalose, which has been shown to preserve the structural integrity of melanoma EVs, decreased the formation of aggregates and increased the fraction of particles within EV size range from 10 to 30% ($p=0.012$). Electroporation buffer (an optimized cell transfection buffer) or trehalose pulse medium was used. Proliferation was measured using carboxyfluorescein succinimidyl ester stain. Electroporation caused a shift in exosome size distribution ($p<0.0001$) and large aggregates by transmission electron microscopy and atomic force microscopy. These studies show the analytical work on ADSC EVs to understand them on a functional level. Thus far, ADSC EVs from diseased patients appear equivalent to healthy ones, and electroporation may optimize their structure without compromising function.

Discussion

Our systematic review of ADSC EV research provides a summary of evidence from numerous clinically applicable animal models of disease. ADSC EVs play multiple roles in ameliorating disease pathologies, stimulating growth, and supporting healing.

Six studies demonstrate the reproducibility of ADSC EV pro-angiogenic abilities, with hypoxic conditions increasing the rate of angiogenesis in two studies.[25,30] The fact that EV treatment rescued function in hypoxic fat grafts due to its miRNA cargo has meaningful implications for the field of plastic surgery, where flap failure is a well-known complication. It may be possible to someday develop proangiogenic EVs to administer in the event of flap failure or even prophylactically. Additionally, the proangiogenic properties seen in the myocardial injury model make a strong argument for further study. Future work may lead to EV therapy for ischemic heart disease.

ADSC EVs were also found, in myriad clinical settings, to rescue cells from apoptosis and attenuate damage after cells were subjected to stress. They were able to reverse apoptosis in cells exposed to hydrogen peroxide, attenuate fibrosis in poisoned liver tissue, rescue engineered Huntington disease cells with programmed genetic mutations, and reduce death of injured myocardial tissue.[30,34,37,43] These studies are evidence of the wide range of abilities that ADSC EVs have, and they represent an important scientific step in developing possible treatments for Huntington disease, cirrhosis, and myocardial infarction.

A primary role of EVs is paracrine function, or the ability to deliver cargo directly to recipient cells. Mechanistic investigations detailed in this review demonstrate that the effects exerted by ADSC EVs are due to this distinct capability. An Alzheimer model demonstrated that EVs deliver

active NEP to target cells.[64] A diabetic foot ulcer study conducted in rats, when subjected to ADSC EVs, promoted the proliferation of endothelial progenitor cells, suggesting a possible future wound healing therapy.[58] EVs have successfully delivered siRNAs to certain cell types in vivo.[65] While the majority of work on EV miRNAs has been done on animal models, the development of disease-specific therapy for humans through targeted delivery of miRNA appears to be on the horizon. Though technology has not yet allowed for manipulation of EV miRNA contents, the invention of one such technique is an exciting conception.

Because the volume of studies on ADSC EVs is small, many avenues for future explorations exist. Artificial means of generating nanovesicles should be further investigated, from both economic and functional standpoints. The aforementioned electroporation, considered as a possible solution to load EVs for drug delivery, produced 30-fold more nanovesicles than natural EVs that also demonstrated equivalent proliferative capacity.[66]

A single study demonstrated that EVs carry the potential to induce apoptosis of cancer cells. This is an exciting prospect that has been investigated outside the realm of adipose stem cells. In fact, non-ADSC EVs have been successfully loaded with doxorubicin and used to inhibit growth of breast and colon cancer cells, demonstrating that this chemotherapeutic delivery is possible.[67,68] Tian et al. used electroporation to load doxorubicin into immature dendritic cell EVs, which resulted in successful delivery to mouse tumors.[67] Subsequent studies could evaluate whether the same techniques could be applied to ADSC EVs.

Future work should also aim to continue characterizing the differences between ADSC EVs from diseased and healthy patients. We have identified one study in which cancer ADSC EVs were determined to be phenotypically identical to healthy counterparts. Continuing studies should be performed to identify any disease-specific characteristics of ADSC EVs.

We acknowledge that systematic reviews of preclinical studies may overestimate positive outcomes, since negative outcomes may less frequently reach publication. We also identify the limitation that all studies included in this review made conclusions based on the cell type tested without screening for adverse events in other organ systems or tissues. Very little is known about the safety of EV therapy, and more work is needed to develop a system of standardized reporting of safety data from these animal studies before proceeding to human subjects. Nevertheless, the results of our systematic review lay down a foundation to support future clinical research on ADSC EVs to repair cellular injury, inhibit tumor growth, and attenuate disease pathologies.

Conclusion

The field of regenerative medicine presents tremendous therapeutic potential. Specifically, ADSCs and their secretome have attracted attention recently due to ease of access and abundance. In this review, ADSC EVs have noted roles in angiogenesis, tissue regeneration, apoptosis, and reduction of cellular damage. While the current existing body of research is still developing, and feasibility studies in humans remain distant, there is a wealth of research possibilities to pursue.

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