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ABOUT COVER

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AIMS AND SCOPE

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The *WJSC* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, PubMed Central, Scopus, Biological Abstracts, BIOSIS Previews, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 Edition of Journal Citation Reports cites the 2021 impact factor (IF) for *WJSC* as 5.247; IF without journal self cites: 5.028; 5-year IF: 4.964; Journal Citation Indicator: 0.56; Ranking: 12 among 29 journals in cell and tissue engineering; Quartile category: Q2; Ranking: 86 among 194 journals in cell biology; and Quartile category: Q2. The *WJSC*'s CiteScore for 2021 is 5.1 and Scopus CiteScore rank 2021: Histology is 17/61; Genetics is 145/335; Genetics (clinical) is 42/86; Molecular Biology is 221/386; Cell Biology is 164/274.

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REVIEW

Clinical trials using dental stem cells: 2022 update

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Abstract

For nearly 20 years, dental stem cells (DSCs) have been successfully isolated from mature/immature teeth and surrounding tissue, including dental pulp of permanent teeth and exfoliated deciduous teeth, periodontal ligaments, dental follicles, and gingival and apical papilla. They have several properties (such as self-renewal, multidirectional differentiation, and immunomodulation) and exhibit enormous potential for clinical applications. To date, many clinical articles and clinical trials using DSCs have reported the treatment of pulpitis, periapical lesions, periodontitis, cleft lip and palate, acute ischemic stroke, and so on, and DSC-based therapies obtained satisfactory effects in most clinical trials. In these studies, no adverse events were reported, which suggested the safety of DSC-based therapy. In this review, we outline the characteristics of DSCs and summarize clinical trials and their safety as DSC-based therapies. Meanwhile, we also present the current limitations and perspectives of DSC-based therapy (such as harvesting DSCs from inflamed tissue, applying DSC-conditioned medium/DSC-derived extracellular vesicles, and expanding-free strategies) to provide a theoretical basis for their clinical applications.

Key Words: Dental stem cells; Adult stem cells; Dental pulp; Tissue regeneration

Core Tip: Since dental pulp stem cells were first isolated and identified in 2000, a variety of dental stem cells (DSCs) have been reported. DSCs have shown satisfactory clinical effects in the treatment of a variety of diseases and have great potential for clinical application. This paper will summarize DSC-based clinical trials and put forward the current limitations and perspectives to accelerate and extend the clinical application of DSCs.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are a population of unspecialized cells characterized by the properties of self-renewal and multidirectional differentiation[1,2]. Currently, MSCs are currently being explored for the treatment of many diseases, such as cardiovascular disease, neurodegenerative diseases, dental diseases, and metabolic diseases[1].

Dental SCs (DSCs) were reported to have similar features to MSCs[3]. Since dental pulp SCs (DPSCs) were first successfully isolated from the extracted third molar in 2000[4], multiple DSC types have been harvested from mature and immature teeth and their surrounding tissues, including periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), stem cells from exfoliated deciduous teeth (SHED), gingiva-derived mesenchymal SCs (GMSCs), and dental follicle progenitor cells (DFPCs)[5-7] (Figure 1). DSCs develop from the neural crest and express both stem cell markers and neural markers[8,9]. It was reported that DSCs have the potential for multipotent differentiation into osteogenic, chondrogenic, adipogenic, neurogenic, odontogenic, dentinogenic cells, and so on[10]. In addition to their self-renewal and differentiation properties, DSCs have also been reported to be involved in secretion, immunomodulation, and tumor processes[3,11]. Based on the characteristics of DSCs, many clinical articles and clinical trials have used DSCs in tissue regeneration and the treatment of various diseases, such as pulpitis, periapical lesions, and periodontitis[12].

In this study, the current status of clinical articles and clinical trials using DSCs in the treatment of various diseases and conditions are reviewed. In addition, current limitations and perspectives, including harvesting DSCs from inflamed tissue, applying DSC-conditioned medium (CM) and DSC-derived extracellular vesicles (EVs), and expanding-free strategies, are also discussed.

CHARACTERISTICS OF DSCS

Based on their various sources, DSCs are divided into DPSCs, SHED, PDLSCs, SCAP, GMSCs, and DFPSCs (Figure 1). DSCs are known to express not only mesenchymal and embryonic stem cell markers (such as CD44, STRO-1, and Nanog) but also neuronal markers because they originate from embryonic neural crests[8,9] (Table 1). However, they do not express CD34, CD45, or CD11b, which are defined as hematopoietic markers[7].

Similar to mesenchymal stem cells, DSCs showed the ability of self-renewal and multidirectional differentiation, such as osteogenic, chondrogenic, adipogenic, neurogenic, odontogenic, dentinogenic, cementogenic, and myogenic differentiation[13-16] (Table 1). In addition, even in the undifferentiated state, DSCs were able to secrete several angiogenic and neurotrophic factors, including vascular endothelial growth factor (VEGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), and β-nerve growth factor (β-NGF), to promote angiogenesis and tissue regeneration[17,18].

In addition, the immunomodulatory features of DSCs have also been the focus of a number of studies. First, it was reported that DSCs, like mesenchymal stem cells, faintly express the MHC class II antigen HLA-DR and maintain low immunogenicity[19-21]. Second, local tissue regeneration and inflammation could be influenced by the secretome of DSCs (including the production of inflammatory and anti-inflammatory cytokines and the regulation of immune cells), which is also regulated by the local inflammatory microenvironment[21-24]. Finally, the inflammatory microenvironment could impact the behaviors of DSCs, such as proliferation potential, migration, homing, and differentiation[22].

Based on the characteristics of DSCs, they have been widely studied in regenerative medicine and tissue engineering and have shown an amazing therapeutic effect on oral-facial, neurologic, corneal, cardiovascular, hepatic, diabetic, renal, muscular, tenogenic, dystrophic and autoimmune conditions in both animal and human models[21,25-27]. For example, the proliferation, paracrine effect, and multidirectional differentiation potential of DSCs support the application of DSCs in regenerative

Table 1 Characteristics of different types of dental stem cells

Cell types	Markers			Multidirectional differentiations
	Cell surface markers	Embryonic stem cell markers	Nerual markers	
DPSCs	CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146, STRO-1[7], CD81, CD49f[140], CD40, CD120a, CD261, CD262, CD264, CD266, CD121a, CD130, CD213a1, CD217, CDw210b[141]	OCT-4, Nanog[142], SSEA-1, SEEA-4[140], SOX-2[143]	βIII-tubulin, NFM, Nestin, CNPase[144], S100, CD271 [17]	Osteogenic, Odontogenic[145], Dentinogeni, Chondrogenic, Neurogenic, Myogenic, Adipogenic[13], Hepatogenic [146]
PDLSCs	CD13, CD29, CD44, CD49, CD73, CD90, CD105, CD146, CD166, CD271 [147], CD10[7], STRO-1[148]	SSEA-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT-4, Nanog, SOX-2, REX1, and ALP[149]	Nestin, OCT-4, SSEA-4[9] CD271, SOX-10[147], SOX-2 [149]	Osteogenic, Cementogenic, Adipogenic, Chondrogenic, Neurogenic[13], Hepatogenic[149], Cardiac myogenic, Endothelial-like, Islet-like, Retinal ganglion-like[147]
SCAP	CD13, CD24, CD29, CD44, CD49, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166, STRO-1, NOTCH-3[150], CD81, CD49f[151]	OCT-4, Nanog, SOX2 [143], CD49f[151]	βIII-tubulin, NFM, Nestin, CNPase[144], SOX-2[143], Vimentin, Survivin[150]	Osteogenic, Dentinogenic, Adipogenic [15], Neurogenic[13], Chondrogenic, Hepatogenic[150]
SHED	CD29, CD73, CD90, CD166[13], STRO-1, CD44[145], CD105[152], NOTCH-1, CD10, CD13, CD34, CD106, CD146, CD166, CD271[102]	OCT-4, Nanog, SSEA-3 [153], SSEA-4[152], NOTCH-1, OCT-4, SOX-2 [102]	βIII-tubulin, NFM, Nestin, CNPase, GAD, NeuN, GFAP [154], CD271, Vimentin, OCT-4, PAX-6, NSE, MAP-2, PSA-NCAM, TH[102]	Osteogenic, Odontogenic[145], Dentinogenic, Chondrogenic, Neurogenic, Myogenic, Adipogenic[13], Hepatogenic[155]
DFPCs	CD13, CD29, CD59, CD90[7], CD105, CD146[142], CD44, CD73, NOTCH-1, STRO-1[156]	OCT-4, Nanog[142], NOTCH-1, SOX-2[156]	OCT-4, SOX2[9], Nestin, SOX-2[156]	Osteogenic, Cementogenic, Odontogenic, Adipogenic, Chondrogenic[13], Hepatogenic[146]
GMSCs	CD13, CD29, CD44, CD73, CD90, CD105, CD146, STRO-1[16]	SSEA-4[16], OCT-4, Nanog[157]	Nestin, SOX10[16], βIII-tubulin, NFM, CNPase[144]	Osteogenic, Adipogenic, Chondrogenic, Neurogenic, Endothelial-like, Odontogenic[16], Myogenic[158]

ALP: Alkaline phosphatase; CD: Cluster of differentiation; CNPase: 2′,3′-cyclic nucleotide 3′-phosphodiesterase; GAD: Glutamic acid decarboxylase; GFAP: Glial fibrillary acidic protein; MAP-2: Microtubule associated protein 2; NeuN: Neuronal nuclei; NFM: Neurofilament medium chain; NGFR: Nerve growth factor receptor; NSE: Neuron-specific enolase; OCT: Octamer-binding transcription factor; PAX-6: Paired Box 6; PSA-NCAM: Polysialylated neural cell adhesion molecule; REX-1: RNA exonuclease 1 homolog; SOX: Sex determining region Y-box; SSEA: Stage-specific embryonic antigen; TH: Tyrosine hydroxylase; SHED: Stem cells from exfoliated deciduous teeth.

medicine (*e.g.*, dental pulp and bone tissue regeneration)[28,29]. The anti-inflammatory, immunomodulatory, and immunoevasive properties of DSCs also help in the treatment of plaque psoriasis[30] (Figure 1). DSC-based therapies have broad prospects for clinical application.

It is worth noting that the naming of mesenchymal stem cells and mesenchymal stromal cells remains controversial. Based on the position paper issued by The International Society for Cell & Gene Therapy (ISCT) Mesenchymal Stromal Cell (ISCT MSC) in 2005, mesenchymal stem cells are not equivalent or interchangeable with mesenchymal stromal cells[31]. Mesenchymal stem cells refer to progenitor cell populations with obvious self-renewal and differentiation functions, while mesenchymal stromal cells refer to large populations with significant secretion, immune regulation, and homing properties[32-34]. As we have just summarized, dental stem cells share some of the characteristics of both mesenchymal stem cells and mesenchymal stromal cells, and more consensus articles may be needed to further define the naming of dental stem cells.

DSC-BASED CLINICAL TRIALS FROM PUBLISHED ARTICLES

Pulpitis and pulp necrosis

Four studies were reported to treat pulp necrosis or irreversible pulpitis using autologous DPSCs or SHED, including a randomized controlled trial (RCT), two case series, and a case report[28,35-37] (Table 2). Xuan *et al*[28] applied SHED in the treatment of pulp necrosis caused by trauma and observed dental pulp tissue regeneration at 12 mo and 24 mo after transplantation. Meanwhile, the results also showed increased dental root length and decreased apical foramen width compared with traditional apexification treatment. Two case series reported by Nakashima *et al*[35,37] indicated that DPSCs transplanted with granulocyte colony-stimulating factor and gelatin sponges could increase pulp sensitivity and mineralization and recover the signal intensity (SI) of regenerated pulp tissue on MRI examination. Meza *et al*[36] transplanted DPSCs and leukocyte platelet-rich fibrin (L-PRF) harvested

Table 2 Dental stem cell-based clinical trials from published articles

Ref.	Registration ID	Conditions/diseases	Study design	Cell source	Administration route	Interventions		Follow-up period	Outcomes
						Test group	Control group		
Xuan *et al*[28], 2018	NCT01814436	Pulp necrosis	RCT	Autologous deciduous pulp	Implanted into injured teeth	SHED (n = 26)	Traditional apexification treatment (n = 10)	12 mo; 24 mo	Dental pulp tissue regeneration; no adverse events observed; the length of the root (↑); the width of the apical foramen (↓)
Nakashima *et al*[35], 2022	None	Irreversible pulpitis	Case series	Autologous dental pulp	Transplanted into the root canal	DPSCs + Gelatin sponge + G-CSF (n = 5)	None	1, 2, 4, 12, 24, 28, 32 wk	Pulp sensibility (↑); MRI examination showed similar SI between test teeth and untreated controls
Nakashima *et al*[37], 2017	None	Irreversible pulpitis	Case series	Autologous dental pulp	Transplanted into the root canal	DPSCs + Gelatin sponge + G-CSF (n = 2)	None	1, 4, 12, 24, and 48 wk	MRI examination showed similar SI between test teeth and untreated controls; mineralized tissue deposition (↑)
Meza *et al*[36], 2019	None	Irreversible pulpitis	A case report	Autologous inflamed dental pulp	Transplanted into the root canal	DPSCs + L-PRF (n = 1)	None	6 mo; 3 year	Delayed response to the cold test; positive response to electric pulp testing; dentin bridge formation
Shiehzadeh *et al*[38], 2014	None	Periapical lesions	Case series	Case 1 and case 3: Autologous apical papilla; case 2: Deciduous pulp	Case 1 and Case 3: Injected from root apex to cavity; case 2: Injected into the defect *via* a surgical approach	Case 1 and Case 3: SCAP + PEG-PLGA scaffold (n = 2); case 2: SHED + PEG-PLGA scaffold (n = 1)	None	Case 1: 30 d, 3 mo, 1 year; 2 year; case 2: 3, 6, 18 mo; case 3: 3, 6, 12, 24 mo	Developed mature apices; periapical tissue healing (↑)
Prasad *et al* [39], 2017	None	Periapical lesions	Case series	Allogeneic deciduous pulp	Transplanted into the root canal	SHED + Bioglass (n = 2)	None	7, 30, 90, 180, 365 d	Closure of open apex; periapical tissue healing; positive response to electric pulp testing and cold testing
Prasad *et al* [40], 2019	None	Periapical lesions	A case report	Allogeneic deciduous pulp	Transplanted into the root canal and periapical area	SHED + Bioglass (n = 1)	None	2 wk; 4, 12, 24 mo	Periapical tissue healing; positive response to electric pulp testing
Ferrarotti *et al* [41], 2018	NCT03386877	Periodontal intrabony defects	RCT	Autologous dental pulp	Implanted into bone defect sites consisted of MIST	Pulp micrografts + Collagen sponge (n = 15)	Collagen sponge (n = 14)	6 and 12 mo	PD (↓); CAL (↓); bone defect fill (↑); residual PD < 5 mm and CAL gain ≥ 4 mm (↑)
Sánchez *et al*	ISRCTN13093912	Periodontal intrabony	CCT	Autologous	Implanted into bone	PDLSCs + β-TCP (n = 9)	β-TCP (n = 10)	1, 3, 6, 9, 12 mo	CAL (-); PPD (-)

[42], 2020		defects		periodontal ligament	defect sites *via* surgical approach				
Feng *et al*[43], 2010	None	Periodontal intrabony defects	Case series	Autologous periodontal ligament	Implanted into bone defect sites *via* surgical approach	PDLPs + HA/TCP (n = 3)	None	3, 6, 12, 32, 42, and 72 mo	CAL (↓); PD (↓); GR (↑)
Chen *et al*[29], 2016	NCT01357785	Periodontal intrabony defects	RCT	Autologous periodontal ligament	Implanted into bone defect sites *via* surgical approach	PDLSCs sheets + DBBM (n = 20)	DBBM (n = 21)	2 wk; 3, 6, 12 mo	CAL (-); PD (-); GR (-)
Iwata *et al*[44], 2018	UMIN000005027	Periodontal intrabony defects	Case series	Autologous periodontal ligament	Implanted into bone defect sites *via* surgical approach	PDL-derived cell sheets + β-TCP (n = 10)	None	3, 6, 55 ± 19 mo	CAL (↓); PD (↓); bone height (↑)
Vandana *et al* [125], 2015	None	Periodontal intrabony defects	A case report	Autologous periodontal ligament	Implanted into bone defect sites *via* surgical approach	Periodontal ligament soft tissue + Gelatin sponge + Cementum scrapings (n = 1)	None	1 wk; 3, 6, 12 mo	CAL (↓); PD (↓); BMD (↑)
Aimetti *et al* [47], 2014	None	Periodontal intrabony defects	A case report	Autologous dental pulp	Implanted into bone defect sites *via* surgical approach	Pulp micrografts + Collagen sponge (n = 1)	None	6 mo; 1 year	PPD (↓); bone fill (↑)
Aimetti *et al* [46], 2018	None	Periodontal intrabony defects	Case series	Autologous dental pulp	Implanted into bone defect sites *via* surgical approach	Pulp micrografts + Collagen sponge (n = 11)	None	1 year	CAL (↓); PD (↓); bone fill (↑)
Aimetti *et al* [49], 2015	None	Periodontal intrabony defects	Case series	Autologous dental pulp	Implanted into bone defect sites *via* surgical approach	Pulp micrografts + Collagen sponge (n = 4)	None	6, 12 mo	PD (↓); CAL (↓); bone fill (↑)
Hernández-Monjaraz *et al* [48], 2018	ISRCTN12831118	Periodontal intrabony defects	A case report	Allogeneic dental pulp	Implanted into bone defect sites *via* surgical approach	DPSCs + Lyophilized collagen-polyvinylpyrrolidone sponge scaffold (n = 1)	None	3, 6 mo	PD (↓); TM (↓); bone fill (↑)
Barbier *et al* [57], 2018	EudraCT database 2014-001913-18	Post-extraction sockets	Split-mouth RCT	Autologous dental pulp	Implanted into postextraction sockets	Pulp micrografts + collagen matrix (n = 30)	Collagen matrix (n = 30)	6 mo	BMD (-); interdental septum height (-)
Cubuk *et al* [62], 2023	NCT04641533	Post-extraction sockets	Split-mouth RCT	Autologous dental pulp	Implanted into postextraction sockets	Pulp micrografts + L-PRF (n = 13)	L-PRF (n = 13)	7 d; 6 mo	PPD (-); CAL (-); vertical bone loss (-); relative bone density (-)
d'Aquino *et al* [58], 2009	None	Post-extraction sockets	Split-mouth CCT	Autologous dental pulp	Implanted into postextraction sockets	Dental pulp stem/progenitor cells + collagen sponge (n = 7)	Collagen sponge (n = 7)	7 d; 1, 2, 3, 12 mo	Rate of mineralization (↑); levels of cortical bone (↑); CAL (↓); BMP-2, VEGF (↑)
Tanikawa *et al* [63], 2020	NCT03766217	Cleft lip and palate	Historical control study	Autologous deciduous pulp	Placed into the alveolar defect *via* surgical approach	SHED + Hydroxyapatite-collagen sponge (n = 6)	rhBMP-2 + Hydroxyapatite-collagen sponge (Group I n = 8); Iliac crest bone graft (Group II n = 8)	6, 12 mo	Bone filling percentage (↑, compared with Group I at the 6-mo follow-up)

Manimaran *et al*[59], 2014	None	Mandibular osteoradionecrosis	A case report	Allogeneic dental pulp	Inserted into the defect after surgical curettage	DPSCs + PRP + TCP (n = 1)	None	2, 6 mo	Bone formation (↑)
Manimaran *et al*[60], 2016	None	Bone defect left by the resection of mandibular ameloblastoma	A case report	Autologous dental pulp	Packed inside the mesh and placed over the mandible after tumor resection	DPSCs + β-TCP + PRF + SVF (n = 1)	None	1, 10 mo; 1.5 years	Bone regeneration (↑); no recurrence of tumor
Brunelli *et al* [61], 2013	None	Sinus lifting	A case report	Autologous dental pulp	Implanted into sinus cavity	Pulp micrografts + Collagen sponge (n = 1)	None	4 mo	BMD (↑)
Koga *et al*[64], 2022	None	Erectile dysfunction	Case series	Allogeneic deciduous pulp	Injected into the penis	SHED-CM (n = 38)	None	After every injection	IIEF-5 score (↑)
Silva *et al*[65], 2022	NCT02728115	Huntington's disease with preexisting pulmonary nodule	A case report	Allogeneic deciduous pulp	Intravenous administrations	SHED (n = 1)	None	15, 30 d; 7, 24, 32 mo	Unified Huntington's disease rating scale (↓); not show long-term tropism or homing for the lung adenocarcinoma
Wang *et al*[93], 2010	None	Plaque psoriasis	A case report	Allogeneic gingival	Bolus injection	GMSCs (n = 1)	None	3 years	Psoriatic lesions fully cleared; no recurrence
Suda *et al*[67], 2022	NCT04608838; JapicCTI194570	Acute ischemic stroke	Study protocol	Allogeneic dental pulp	Intravenous administration	DPSCs	Placebo	Per 15 min (1-4 h); per 30 min (4-6 h); 12, 24 h; 2, 3, 8, 31, 91, 181, 366 d	No results
Nagpal *et al* [68], 2016	None	Chronic disability after stroke	Study protocol	Autologous dental pulp	Implanted into peri-infarct region *via* neurosurgical procedure	DPSCs	None	1, 6, 9, 12 mo	No results
Ye *et al*[69], 2020	ChiCTR2000031319: NCT04336254	COVID-19	Study protocol	Allogeneic dental pulp	Intravenous administration	DPSCs	Saline	2 h ± 30 min; 24 h ± 30 min; 90 d ± 3 d	No results

BMD: Bone mineral density; CAL: Clinical attachment level; CCT: Controlled clinical trials; COVID-19: Coronavirus disease 2019; DBBM: Deproteinized bovine bone mineral; GR: Gingival recession; G-CSF: Granulocyte colony stimulating factor; HA/TCP: Hydroxyapatite/tricalcium phosphate; IIEF: International index of erectile function; L-PRF: Leukocyte-platelet rich fibrin; MIST: Minimally invasive surgical technique; MRI: Magnetic resonance imaging; PD: Probing depth; PDL: Periodontal ligament; PDLPs: Periodontal ligament progenitor cells; PPD: Periodontal probing depth; PRF: Platelet rich fibrin; PRP: Platelet-rich plasma; PEG-PLGA: Poly (lactide-co glycolide)-polyethylene glycol; RCT: Random clinical trial; rh-BMP: Recombinant human bone morphogenetic protein; SI: Signal intensity; SVF: Stromal vascular fraction: TCP: Tricalcium phosphate; TM: Tooth mobility; VEGF: Vascular endothelial growth factor; SHED-CM: Stem cells from exfoliated deciduous teeth conditioned medium.

from autologous inflamed dental pulp and blood, respectively, to the root canal of irreversible pulpitis teeth and observed dentin bridge formation and a response to the cold test and electric pulp test.

Periapical lesions

In a case report and two case series, SCAP/SHED combined with a polyethylene glycol polylactic-polyglycolic acid (PEG-PLGA) scaffold and SHED combined with bioglass were used for the treatment of periapical lesions[38-40] (Table 2). Periapical tissue healing was found in the follow-up examinations

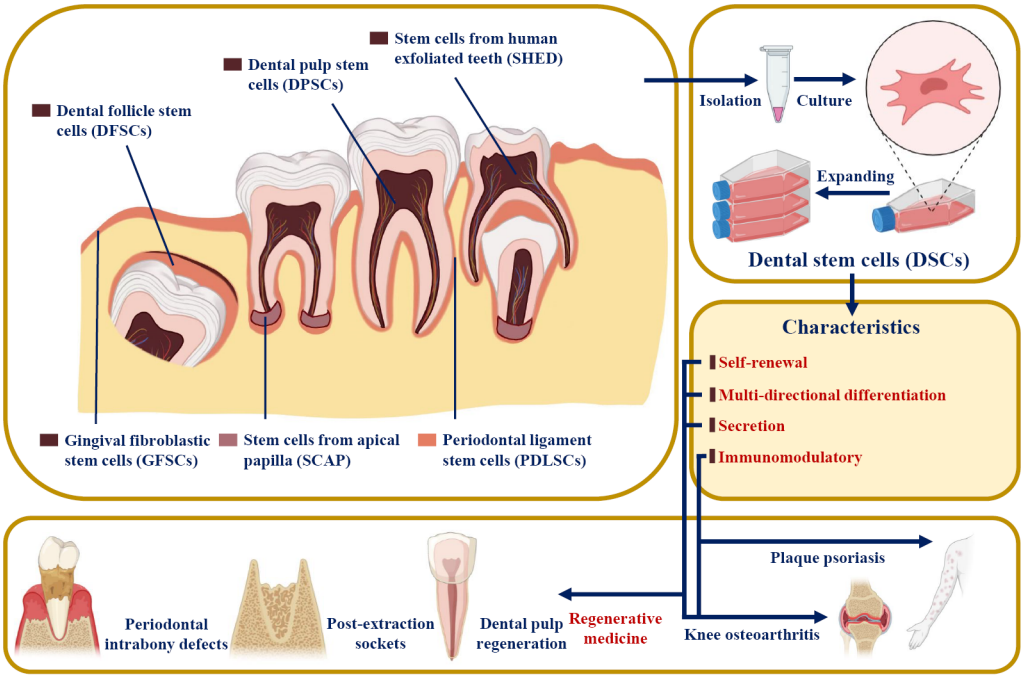

Figure 1 Tissue origin, harvest, characteristics, and clinical application potential of the various populations of dental stem cells. Dental pulp stem cells and stem cells from exfoliated deciduous teeth can be isolated from the inner dental pulp of permanent teeth and deciduous exfoliated teeth, respectively. Stem cells from apical papilla can be extracted from the apical papilla; periodontal ligament stem cells can be harvested from the periodontal ligament; and dietary fiber supplementation combinations can be derived from the dental follicle. Gingiva-derived mesenchymal stem cells can be extracted from gingiva. Citation: Sharpe PT. Dental mesenchymal stem cells. *Development* 2016; 143: 2273-2280[139]. Copyright ©The Authors 2016. Published by The Company of Biologists Ltd. The authors have obtained the permission for figure using from the Company of Biologists Ltd (Supplementary material).

of all three studies. It was reported a positive response in the test of dental pulp activity after SHED transplantation, suggesting the regeneration of pulp or pulp-like tissue, which does not occur in traditional root canal therapy[39,40].

Periodontal intrabony defects

There are two RCTs, a controlled clinical trial (CCT), three case series, and two case reports of DSC-based treatment for periodontal intrabony defects[29,41-46] (Table 2). The RCT of Ferrarotti *et al*[41] indicated that pulp micrografts applied with collagen sponges could significantly reduce PD and CAL and promote the regeneration of bone defects when compared with collagen sponges alone. Three case series and a case report using pulp micrografts/DPSCs and collagen sponges also reported similar results of periodontal benefits[46-49]. It was reported a novel approach using periodontal ligament soft tissue, gelatin sponges, and cementum scrapings, which reduced the CAL and PD of periodontitis teeth in their case report[45].

Although two case series demonstrated the periodontal benefits of PDLPs and PDL-derived cell sheets[43,44], significant differences in periodontal indices (including PD and CAL) were not observed between the test groups and control groups in the other two CCTs that applied PDLSC and PDLSC sheets[29,42]. Several factors might have contributed to the lack of significant differences in the outcomes, such as satisfactory scaffold material properties and small sample sizes. In these four studies, β-TCP, HA/TCP, and deproteinized bovine bone mineral (Bio-oss®) were applied as scaffold materials. Although some studies reported abilities to provide support for PDLSCs on osteogenic differentiation of these scaffolds*in vitro* and *in vivo*[50-53], only using these scaffolds also achieved great clinical benefits in the treatment of periodontitis[54-56]. The excellent performance of the scaffold may have overshadowed the contribution by PDLSCs. More clinical studies at multiple centers with different amounts and types of DSCs, more follow-up time points, and larger sample sizes are necessary, and the results of such studies would be meaningful.

Bone defects caused by other conditions

In addition to periodontal intrabony defects, DSCs were also used for the treatment of post-extraction sockets, mandibular osteoradionecrosis, bone defects after ameloblastoma resection, and sinus lifting [57-61] (Table 2). Two split-mouth RCTs reported by Barbier *et al*[57] and Cubuk *et al*[62] did not find significant differences in BD or interdental septum height between the pulp micrograft + scaffold (collagen matrix/L-PRF) group and the scaffold (collagen matrix/L-PRF) group after implantation into post-extraction sockets. However, in another split-mouth CCT designed for regenerating post-extraction

sockets, DPSCs combined with collagen sponges promoted the rate of mineralization, the levels of cortical bone, and the expression of bone morphogenetic protein-2 (BMP-2) and VEGF when compared with collagen sponge treatment alone[58]. Tanikawa *et al*[63] reported a historical control study comparing the effects of SHED, rhBMP, and iliac crest bone grafts in treating cleft lip and palate. The SHED group showed similar satisfactory performance in bone healing compared with iliac crest bone grafts and a higher bone filling percentage compared with the rhBMP group at the 6-mo follow-up[63].

Two case reports indicated that DPSCs combined with TCP could increase the bone regeneration of bone defects caused by osteoradionecrosis and ameloblastoma[59,60]. A case report by Brunelli *et al*[61] demonstrated that pulp micrografts + collagen sponges increased the BD in newly formed bone when applied for sinus lifting.

Other conditions

Koga *et al*[64] reported a case series that applied SHED conditioned medium (SHED-CM) to treat erectile dysfunction. In this study, the international index of erectile function (IIEF-5), which is clinically used to screen for erectile function and to assess treatment efficacy, was increased after SHED-CM injection into the corpus cavernosum of erectile dysfunction patients[64]. A case report indicated that SHED intravenous administrations could decrease the scale of unified Huntington's disease rating, which is designed to assess clinical performance and capacity in patients with Huntington's disease[65, 66]. Meanwhile, the patient with Huntington's disease also suffered from preexisting pulmonary nodules, and SHED injection did not result in long-term tropism or homing for the patient's lung adenocarcinoma[65]. In a case report by Wang *et al*[30], GMSCs were used to treat plaque psoriasis *via* bolus injection, and they observed fully cleared psoriatic lesions without recurrence.

Three clinical study protocols using DSCs have been published in recent years, including the treatment of acute ischemic stroke, chronic disability after stroke, and COVID-19[67-69].

DSC-BASED CLINICAL TRIALS FROM CLINICAL DATABASES

ClinicalTrials.gov (https://clinicaltrials.gov/) and the International Clinical Trials Registry Platform (ICTRP, https://trialsearch.who.int/) were screened for DSC-based clinical trials.

To date, there have been 21 clinical trials registered on ClinicalTrials.gov evaluating the use of DSCs in treating periodontitis (33.3%, 7/21), post-extraction sockets (4.8%, 1/21), edentulous alveolar ridge (4.8%, 1/21), cleft lip and palate (9.5%, 2/21), knee osteoarthritis (4.8%, 1/21), dental pulp necrosis (4.8%, 1/21), liver cirrhosis (4.8%, 1/21), type 1 diabetes (4.8%, 1/21), acute ischemic stroke (4.8%, 1/21), Huntington's disease (14.3%, 3/21), and COVID-19 (9.5%, 2/21) (Table 3). In addition to the 6 studies reported in ClinicalTrials.gov, 7 clinical trials were registered on the ICTRP using DSCs in the treatment of periodontitis (57.1%, 4/7), wrinkles (28.6%, 2/7), and hair loss (14.3%, 1/7) (Table 4). In all, 28 clinical trials were registered on these two platforms.

Several registered clinical trials applied two stages in one work. The most frequently appearing trial phases were phase 1 (42.9%, 12/28), followed by phase 2 (25%, 7/28), Phase 3 (7.1%, 2/28), and Phase 0 (3.6%, 1/28). There were 10 trials (35.7%) in which the phase design was not applied or not selected. One clinical trial reported the outcomes both on the registry platform and in a published article[63] (NCT01932164), and the published articles of seven trials stated the registered ID[29,42,62,63,67,69-71], while other trials did not publish any data.

Consistent with the literature, the proportion of clinical trials using DSCs to treat periodontitis was the highest. Eleven registered clinical trials researched the effect of DSCs on periodontitis (39.3%, 11/28). In these trials, various amounts, types, and injection times of DSCs and different application modes (such as DSCs, micrografts, cell sheet pellets, and cell sheet fragments) were applied. In addition, several scaffolds were used in combination with DSCs, including collagen sponges, deproteinized bovine bone minerals, β-TCP scaffolds, and hydroxyapatite-collagen scaffolds.

SAFETY ISSUES REGARDING DSC-BASED THERAPY

Although encouraging treatment effects on diseases have been achieved, the safety issues of stem cell-based therapy remain controversial, especially in long-term follow-up[72]. At present, the limitations of stem cell-based therapy are mainly focused on non-directional differentiation, accelerating tumor progression.

In addition, uncontrolled non-directional differentiation may have a great impact on the safety of stem cell transplantation. Breitbach *et al*[73] found that the encapsulated structures in the infarcted areas contained calcifications and/or ossifications in myocardial infarction mice after MSC injection. In another study, unselected bone marrow cells injected directly induced significant intramyocardial calcification in acutely infarcted myocardium[74].

Table 3 Dental stem cell-based clinical trials registered at clinicaltrials.gov

Ref.	Registration ID	Status	Diseases	Study design	Cell source	Administration route	Number of patients	Interventions		Follow-upperiod	Phase	Outcomes
								Test group	Control group			
-	NCT04983225	Recruiting	Periodontitis	Randomized; parallel assignment; double-blind (participant, investigator)	Dental pulp	Injecting into the periodontal defect site	36	DPSCs (1×10^6)/site; DPSCs (5×10^6)/site; DPSCs ($3\text{-}4 \times 10^7$)/three or four sites; DPSCs (1×10^7)/site; DPSCs (2×10^7)/two sites	Saline solution	90, 180, 360, 720 d	Phase 1	
-	NCT02523651	Unknown	Periodontitis	Randomized; parallel assignment; triple-blind (participant, investigator, outcomes Assessor)	Allogeneic dental pulp	Injecting into the periodontal defect site	40	DPSCs (1×10^6)	Saline solution	1 year	Phase 1/2	
-	NCT03386877	Completed	Periodontitis	Randomized; parallel assignment; triple-blind (participant, investigator, outcomes assessor)	Autologous dental pulp	Delivering into intrabony defect *via* minimally invasive surgical technique	29	Micrografts of DPSCs + Collagen sponge	Collagen sponge	6, 12 mo	Not applicable	
-	NCT01082822	Unknown	Periodontitis	Nonrandomized; parallel assignment; open label	Periodontal ligament	Implanted into bone defect sites *via* surgical approach	80	PDLSCs sheet fragment + DBBM (Bio-oss); PDLSCs sheet pellets + DBBM (Bio-oss); DBBM (Bio-oss)	Sham comparator	4, 12, 24 wk; 1 year	Phase 1/2	
-	NCT03638154	Completed	Periodontitis	Randomized; parallel assignment; double-blind (care provider, outcomes assessor)	Gingival	Implanted into bone defect sites *via* surgical approach	20	GFs + GMSCs + β-TCP	β-TCP	1, 3, 7, 14 d; 6 mo	Not applicable	
-	NCT03137979	Unknown	Periodontitis	Randomized; parallel assignment	Gingival	Implanted into bone defect sites *via* surgical approach	30	GMSCs + Collagen scaffolds; collagen scaffolds	Open flap debridement	1, 3, 6 mo	Phase1/2	
Chen *et al* [29], 2016	NCT01357785	Unknown	Periodontitis	Randomized; parallel assignment; open label	Autologous periodontal ligament		35		None	3-12 mo	Phase1	
Cubuk *et al* [62], 2023	NCT04641533	Completed	Post-extraction sockets	Split-mouth; randomized; crossover assignment; double-blind (investigator, outcomes assessor)	Dental pulp	Placing into the extraction socket	13	DPSCs + L-PRF	L-PRF	7 d; 6 mo	Not applicable	
-	NCT02731586	Unknown	Edentulous alveolar ridge	Single group assignment; open label	Allogeneic dental pulp	Introducing dental pulp-derived mesenchymal stem cells during placement of dental implants	10	Dental pulp-derived MSCs	None	3 mo	Early Phase 1	

Tanikawa *et al*[63], 2020; Pinheiro *et al* [70], 2019	NCT03766217	Completed	Cleft lip and palate	Randomized; parallel assignment; single-blind (outcomes assessor)	Autologous deciduous pulp	Placed into the alveolar defect *via* surgical approach	62	SHED + Hydroxyapatite-collagen sponge	Iliac crest autogenous bone graft	15 d; 3, 6, 12 mo	Phase3	
Tanikawa *et al*[63], 2020	NCT01932164	Completed; Has results	Cleft lip and palate	Single group assignment; open label	Autologous deciduous pulp	Maxillary alveolar graft by tissue engineering	5	SHED + Hydroxyapatite-collagen sponge	None	3, 6 mo	Not applicable	Percentage of bone filling at 6 mo postoperatively: 89.5%
-	NCT04130100	Unknown	Knee osteoarthritis	Randomized; parallel assignment; open label	Dental pulp	Intraarticular injection	60	Low dose of DPSCs; high dose of DPSCs	Sodium hyaluronate	12 mo	Early phase 1	
-	NCT01814436	Unknown	Dental pulp necrosis	Single group assignment;open label	Autologous deciduous pulp		80	Scaffold-free SHED-derived pellet	None	3-12 mo	Not applicable	
-	NCT03957655	Unknown	Liver cirrhosis	Randomized; parallel assignment; single-blind (outcomes assessor)	Autologous deciduous pulp	Peripheral vein infusion	40	SHED (1×10^6 cells/kg body weight)	Standard medication for viral hepatitis and cirrhosis	4, 8, 12, 16, 24 wk	Early phase 1	
-	NCT03912480	Unknown	Type 1 diabetes	Single group assignment; open label	Deciduous pulp	Intravenous drip	24	SHED (0.11 IU/kg body weight) + Insulin + oral hypoglycemic drugs	None	1, 2, 6 wk; 2, 3, 6, 9, 12 mo	Early phase 1	
Suda *et al* [67], 2022	NCT04608838	Completed	Acute ischemic stroke	Randomized;Parallel assignment;Quadruple-blind (Participant, Care Provider, Investigator, Outcomes Assessor);	Allogeneic dental pulp	Intravenously infusion	79	DPSCs (JTR-161, 1×10^8 cells); DPSCs (JTR-161, 3×10^8 cells)	Placebo	91, 366 d	Phase 1/2	
-	NCT02728115	Active, not recruiting		Nonrandomized; parallel assignment; open label	Allogeneic deciduous pulp	Intravenous administration	6	SHED (Cellavita HD, 1×10^6 cells); SHED (Cellavita HD, 2×10^6 cells)	None	1, 4 years	Phase 1	
-	NCT04219241	Active, not recruiting	Huntington's disease	Single group assignment; open label	Allogeneic deciduous pulp	Intravenous administration	35	SHED (Cellavita HD, 2×10^6 cells)	None	1, 2 years	Phase 2/3	
Wenceslau *et al*[71], 2022	NCT03252535	Completed	Huntington's disease	Randomized; parallel assignment; triple-blind (participant, investigator, outcomes assessor)	Allogeneic deciduous pulp	Intravenous administration	35	SHED (Cellavita HD, 1×10^6 cells); SHED (Cellavita HD, 2×10^6 cells)	Physiological solution without cells	Monthly for 14 mo	Phase 2	
Ye *et al*[69], 2020	NCT04336254	Recruiting	COVID-19	Randomized; parallel assignment; triple-blind (participant, investigator, outcomes assessor)	Allogeneic dental pulp	Intravenous injection	20	DPSCs (3×10^7 cells)	Saline	28 d	Phase 1/2	
-	NCT04302519	Unknown	COVID-19	Single group assignment; open label	Dental pulp	Intravenous injection	24	DPSCs (1×10^7 cells/kg body weight)	None	3, 7, 14, 28, 360 d	Early phase 1	

DBBM: Deproteinized bovine bone mineral; GFs: Gingival fibroblast; PRF: Platelet-rich fibrin; TCP: Tricalcium phosphate; DPSCs: Dental pulp stem cells; SHED: Stem cells from exfoliated deciduous teeth.

Similar to the regeneration of damaged tissue, tumors exert chemotactic effects on MSCs, affecting their recruitment to tumor sites[75-77]. Current studies have shown that MSCs have bidirectional, anti-cancer and pro-cancer, regulatory effects, which raises safety concerns for clinical application. On the one hand, MSCs are the major component of the tumor microenvironment and can be reprogrammed to the pro-tumorigenic phenotype by the tumor[78]. MSCs have been revealed to participate in the initiation, development, progression, and metastasis of multiple cancers[79]. The pro-cancer effect of stem cells may be achieved by secreting molecules that affect the phenotype of tumor cells, promoting tumor angiogenesis, cancer-associated fibroblast differentiation, cell-to-cell contact, or cell engulfment [76]. In recent studies, DPSCs and their conditioned medium were reported to promote the proliferation and carcinogenic properties of prostate cancer, oral cancer, breast cancer, and melanoma cells*in vitro*[80-82].

On the other hand, there is also evidence that MSCs can inhibit the growth of a variety of tumors, including breast cancer, Kaposi's sarcoma, hepatoma, glioma, and melanoma[76,83-85]. DPSCs and their conditioned medium also showed a suppressive effect on the development and migration of colorectal cancer cells through mitogen-activated protein kinase pathways[86]. In fact, there are few reports of primary pulp malignancies[87]. In a genome-wide RNA-seq study, phosphatase and tensin homolog (PTEN) expression in DPSCs was higher than that in BMSCs[88]. PTEN, a phosphatase, can metabolize phosphatidylinositol 3,4,5-triphosphate and directly oppose the activation of the oncogenic PI3K/AKT/mTOR signaling network[89]. At present, the regulatory effects of stem cells on cancer are still controversial, and the difference in results may be related to cell lines, cell doses, animal models, cancer types, treatment duration time, and other factors.

In conclusion, no adverse events were reported in the published clinical articles or clinical trials using DSCs, which suggested the safety of DSC-based therapy. However, based on current concerns about the safety of stem cell therapy, more *in vivo* studies on the safety of DSC-based therapies are of great significance.

CURRENT LIMITATIONS AND PERSPECTIVES

Harvesting DSCs from inflamed tissue

Most studies applied stem cells extracted from healthy dental tissue for treatment, but additional surgery (such as third molar extraction) might increase patient suffering. Harvesting stem cells from inflamed dental tissue could be an alternative method, although stem cell abilities might be affected[36,90,91].

Several studies have researched the different biological properties of DPSCs derived from normal and inflamed pulps (iDPSCs), and the results are still in dispute[92-98]. In some studies, DPSCs showed better self-renewal ability[92,93] and multidirectional differentiation capacities than iDPSCs[92], while in other studies, no significant difference was observed[94,95,98]. A study by Nie *et al*[97] indicated that DPSCs showed higher colony-forming, proliferative, and osteo/dentinogenesis abilities, while iDPSCs

Table 4 Dental stem cell-based clinical trials registered on the International Clinical Trials Registry Platform

Ref.	Registration ID	Status	Diseases	Study design	Cell source	Administration route	Number of patients	Interventions		Follow-up period	Phase	Outcomes
								Test group	Control group			
-	JPRN-UMIN000042791	Complete: Follow-up complete	Periodontitis	Randomized; parallel assignment; single-blind (participants)	Deciduous pulp	Gargle	30	Mouthwash containing SHED culture supernatant	Mouthwash without SHED culture supernatant	1 mo	Not applicable	
-	ChiCTR2100051466	Recruiting	Periodontitis	Randomized; parallel assignment; open label	Dental pulp	Bilateral multipoint injection on a single tooth	96	DPSCs (1×10^7 cells) for once; DPSCs (1×10^7 cells) for twice	Saline	90, 180, 360 d	Phage 0	
-	ChiCTR2100049178	Pending	Periodontitis	Randomized; parallel assignment; double-blind	Dental pulp	Local injection	36	DPSCs (1×10^6 cells) for single injection; DPSCs (5×10^6 cells) for single injection; DPSCs (1×10^7 cells) for single injection; DPSCs (1×10^7 cells) for single injection in 2 locations; DPSCs (1×10^7 cells) for single injection in 3-4 locations	None		Phage 1	
Sánchez *et al* [42], 2020	ISRCTN13093912	Completed	Periodontitis	Randomized; parallel assignment; single-blind (patients and examiners)	Dental pulp	Implanted into bone defect sites *via* surgical approach	20	DPSCs (1×10^7 cells) + hydroxyapatite-collagen scaffold	Hydroxyapatite-collagen scaffold	1, 2, 4, 12, 24, 36 wk; 12, 24, 36, 48, 60 mo	Not applicable	
-	JPRN-UMIN000045926	Complete: Follow-up complete	Wrinkles	Randomized; parallel assignment; single-blind (outcomes assessor)	Dental pulp		12	All-in-one gel containing immortalized DPSCs-CM solution and various beauty ingredients	No treatment	4 wk	Not applicable	
-	JPRN-UMIN000043528	Complete: Follow-up complete	Wrinkles	Randomized; parallel assignment; single-blind (outcomes assessor)	Dental pulp		12	All-in-one gel containing immortalized DPSC-CM solution and the latest peptide raw materials	No treatment	4 wk	Not applicable	
-	JPRN-UMIN000045897	Complete: Follow-up continuing	Hair loss	Nonrandomized; parallel assignment; open label	Deciduous pulp	Injection	22	SHED-CM; after SHED-CM injection, one dose of micrografts (Rigenera) followed by another SHED-CM injection; SHED-CM injection after one dose of micrografts (Rigenera)	None	6 mo	Not applicable	

DPSCs: Dental pulp stem cells; SHED-CM: Stem cells from exfoliated deciduous teeth conditioned medium.

demonstrated enhanced chondrogenesis, neurogenesis, angiogenesis, and adipogenesis capacities. Park *et al*[96] reported that iDPSCs appear to have higher osteogenic differentiation potential and lower neurogenic differentiation potential than DPSCs.

Differences in inflammation levels may explain the discrepancy in the biological properties of DPSCs and iDPSCs in various studies. Intense and rapid inflammatory stimulation irreversibly initiates pulp necrosis, while low insult levels of inflammation are able to cause reversible pulpitis and promote dentine regeneration[99]. DPSCs are a suitable source of stem cells for pulp nerve regeneration because of their neuronal differentiation potential. It was reported that acute inflammation with a high level of proinflammatory cytokines could reduce neural precursor cell (NPC) survival and inhibit the neuronal differentiation of NPCs, while chronic inflammation expressed a potentially neuroprotective phenotype and supported neuronal differentiation[100]. Meanwhile, age, sex, tooth position, and sample size are also confounding factors affecting the function of DPSCs, which should be considered in subsequent studies and clinical practice.

DSC-CM and DSC-EVs

The culture medium collected from cells in culture is known as CM. CM is applied as an alternative therapy for tissue regeneration, which is a less ethical issue because it uses cells indirectly. Koga *et al*[64] applied SHED-CM in the treatment of erectile dysfunction, which is the only record of its clinical use to the best of our knowledge.

DSC-CM contains a variety of cytokines associated with vascular and nerve tissue regeneration, such as VEGF, BDNF, β-NGF, GDNF and neurotrophin-3 (NT-3)[101,102]. To date, DSC-CM has been reported to have the potential to promote bone regeneration[103], periodontal regeneration[104], angiogenesis[105], pulp regeneration[106], and nerve protection/regeneration[105,107-109] with great possibilities for clinical application.

In addition, DSC-CM showed satisfactory anti-inflammatory and immunoregulatory effects. Several *in vivo* studies based on various animal models reported that intravenous injection or intranasal administration of SHED-CM improved liver fibrosis[110], acute liver failure[111], acute lung injury [112], Alzheimer's disease, temporomandibular joint osteoarthritis[113], Sjögren's syndrome[114], and rheumatoid arthritis[115] by exerting anti-inflammatory effects. Meanwhile, studies have also reported the effect of SHED-CM on promoting Treg cell differentiation[114] and M2-like macrophage induction [111,112], as well as inhibiting Th17 cell differentiation[114] and inflammatory macrophage activation [116].

In addition to DSC-CM, DSC-EVs harvested from cell-culture medium have also been deeply studied in recent years. Multiple studies have indicated the promotion effect of DSC-EVs on jawbone and calvarial bone regeneration[117,118], angiogenesis and cutaneous wound healing *in vivo*[119,120]. Li *et al* [121] also reported that DSC-EVs could alleviate cerebral ischemia-reperfusion by suppressing the inflammatory response, which is related to the inhibition of the HMGB1/TLR4/MyD88/NF-κB pathway.

The poor survival rate of implanted DSCs and host immunogenic reactions are the main drawbacks of applying DSCs directly. In some comparative studies, stem cell-derived CM showed similar and even better treatment effects on acute lung injury, Parkinsonism, and type 1 diabetes than the direct use of stem cells[112,122,123]. DSC-CM and its components (such as EVs) provide several key advantages over cell-based applications, including avoiding the risk of host immunogenic reactions, cost-effectiveness, long-term storage capacity, and simpler evaluation of safety and efficacy[104,124]. Accumulating evidence indicates the great potential of DSC-CM/DSC-EV-based treatment in clinical applications.

Expanding-free strategy

Despite encouraging results of differentiation and tissue regeneration, DSCs still require rigorous cell-expanding procedures to obtain a sufficient number of cells for treatment, which is costly with great technique sensitivity, often taking tens of days. The *ex vivo* expansion of stem cells often reduces their self-renewal and proliferation abilities[125]. Direct mechanical digestion or tissue transplantation are promising solutions to these limitations.

In recent years, using mechanical disaggregation of dental tissues instead of cell-expending procedures was successful for harvesting autologous pulp micrografts rich in progenitor cells[41,126]. In 2016, Monti *et al*[126] indicated that DSCs harvested by mechanical digestion (Rigenera® system, HBW, Turin, Italy) were fully comparable to stem cells obtained after enzymatic digestion. In this study, mechanical digestion-obtained DPSCs showed osteogenic, adipogenic, and chondrogenic differentiation abilities*in vitro* and were able to increase the regeneration of post-extraction sockets *in vivo* when applied with the collagen sponge[126].

Pulp micrografts harvested by mechanical digestion were also applied in the treatment of sinus lifting, post-extraction sockets, and periodontal intrabony defects[46,47,49,57,61,62]. One clinical trial using pulp micrografts was also designed for periodontitis management (NCT03386877), but the outcome was not reported. Different systems of mechanical disaggregation were applied in these studies, including BD Medimachine (BD Biosciences San Jose, CA, United States)[62], the Rigenera® system (HBW, Turin, Italy)[46,57,61], and the Medimachine System (Consul TS, Orbassano, Italy)[47, 49]. In brief, dental pulp is first collected from extracted teeth and then sent to the mechanical disaggregation system to obtain pulp micrografts. After filtration or without filtration, pulp micrografts are combined with the scaffold for transplantation.

In addition, Vandana *et al*[125] described a novel approach using stem cell assistance in the periodontal regeneration technique (SAI-PRT), which contained periodontal ligament soft tissue gelatin sponge scaffolds and cementum scrapings. In their research, SAI-PRT successfully bypassed*in vitro* culture and expanded PDLSCs, resulting in satisfactory defect filling of periodontal intrabony defects [125].

Embryonic stem cells, PSCs, and DSCs

Embryonic stem cells (ESCs) are pluripotent cells of great significance to developmental biology. They give rise to all types of germ layer cells in the embryo. The self-renewal ability and plasticity of ESCs make it possible to generate unlimited numbers of different types of cells *in vitro*[127]. Similar to embryonic cells, PSCs derived from different somatic cells also have the ability to immortalize and differentiate into the three germ layers[128]. The properties of these two cell types make them promising sources for stem cell-based therapy for various diseases and injuries. However, due to the limitations of ESCs and PSCs, adult stem cells (such as DSCs) still possess high application value.

First, ethical issues regarding the use of ESCs make their clinical application challenging[128]. Second, the preparation of autologous PSCs takes a long time (more than 3 mo) and has high medical cost, and the immune rejection issue of allotransplantation should be considered[129]. In addition, teratomas are germ cell tumors containing cells of two or three germ lines that always occur *via* uncontrollable stem cell proliferation and differentiation[130,131]. In experimental studies, stem cell transplants (especially ESC and PSC transplants) have been found to increase the risk of teratomas, raising safety concerns[131-133]. Previously, viral vector integration and contamination of animal-derived components also posed obstacles to the use of PSCs, but these problems have been addressed by innovative techniques, such as integration-free methods and xeno-free culture[134-136].

DSCs did not show unlimited proliferation potential and demonstrated poorer differentiation ability than PSCs and ESCs[137]. However, the advantages of DSCs over ESCs and PSCs, such as fewer ethical issues and lower teratoma risk[87,88,138], lower cost and shorter preparation period, harvesting from medical waste, and implementing therapeutic effects without gene editing, grant them greater potential for clinical applications in the future.

CONCLUSION

Many clinical articles and clinical trials of autologous and allogeneic DSCs have aimed to evaluate their therapeutic effects on various diseases, such as pulpitis, periapical lesions, periodontitis, cleft lip and palate and Huntington's disease. In most studies, satisfactory clinical treatment results were obtained, while clinical benefits of using DSCs were not found in some research. Although safety risks exist for stem cell-based therapies, safety issues have not been reported in the clinical applications of DSCs. In the future, in addition to continuing to study the efficacy and safety of DSC-based treatment, harvesting DSCs from inflammatory tissues, expanding-free strategies, and applying DSC-CM or DSC-EVs should be studied, as they have strong research value and application potential. Taken together, DSC-based therapy is a promising tool for the treatment of various diseases and can be further promoted.

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FOOTNOTES

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REFERENCES

1 **Zakrzewski W**, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther* 2019; **10**: 68 [PMID: 30808416 DOI: 10.1186/s13287-019-1165-5]
2 **Kolios G**, Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration* 2013; **85**: 3-10 [PMID: 23257690 DOI: 10.1159/000345615]
3 **Gan L**, Liu Y, Cui D, Pan Y, Zheng L, Wan M. Dental Tissue-Derived Human Mesenchymal Stem Cells and Their Potential in Therapeutic Application. *Stem Cells Int* 2020; **2020**: 8864572 [PMID: 32952572 DOI: 10.1155/2020/8864572]
4 **Gronthos S**, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and *in vivo* . *Proc Natl Acad Sci U S A* 2000; **97**: 13625-13630 [PMID: 11087820 DOI: 10.1073/pnas.240309797]
5 **Mizisin AP**, Wiley CA, Hughes RA, Powell HC. Peripheral nerve demyelination in rabbits after inoculation with Freund's complete adjuvant alone or in combination with lipid haptens. *J Neuroimmunol* 1987; **16**: 381-395 [PMID: 2444628 DOI: 10.14670/hh-29.699]
6 **Mai Z**, Chen H, Ye Y, Hu Z, Sun W, Cui L, Zhao X. Translational and Clinical Applications of Dental Stem Cell-Derived Exosomes. *Front Genet* 2021; **12**: 750990 [PMID: 34764982 DOI: 10.3389/fgene.2021.750990]
7 **Aydin S**, Şahin F. Stem Cells Derived from Dental Tissues. *Adv Exp Med Biol* 2019; **1144**: 123-132 [PMID: 30635857 DOI: 10.1007/5584_2018_333]
8 **Heng BC**, Jiang S, Yi B, Gong T, Lim LW, Zhang C. Small molecules enhance neurogenic differentiation of dental-derived adult stem cells. *Arch Oral Biol* 2019; **102**: 26-38 [PMID: 30954806 DOI: 10.1016/j.archoralbio.2019.03.024]
9 **Bonaventura G**, Incontro S, Iemmolo R, La Cognata V, Barbagallo I, Costanzo E, Barcellona ML, Pellitteri R, Cavallaro S. Dental mesenchymal stem cells and neuro-regeneration: a focus on spinal cord injury. *Cell Tissue Res* 2020; **379**: 421-428 [PMID: 31776822 DOI: 10.1007/s00441-019-03109-4]
10 **Nuti N**, Corallo C, Chan BM, Ferrari M, Gerami-Naini B. Multipotent Differentiation of Human Dental Pulp Stem Cells: a Literature Review. *Stem Cell Rev Rep* 2016; **12**: 511-523 [PMID: 27240827 DOI: 10.1007/s12015-016-9661-9]
11 **Zhai Q**, Dong Z, Wang W, Li B, Jin Y. Dental stem cell and dental tissue regeneration. *Front Med* 2019; **13**: 152-159 [PMID: 29971640 DOI: 10.1007/s11684-018-0628-x]
12 **Yamada Y**, Nakamura-Yamada S, Konoki R, Baba S. Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine. *Stem Cell Res Ther* 2020; **11**: 175 [PMID: 32398041 DOI: 10.1186/s13287-020-01683-x]
13 **Huang GT**, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009; **88**: 792-806 [PMID: 19767575 DOI: 10.1177/0022034509340867]
14 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736 [PMID: 19172693 DOI: 10.1038/nri2395]
15 **Sonoyama W**, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008; **34**: 166-171 [PMID: 18215674 DOI: 10.1016/j.joen.2007.11.021]
16 **Kim D**, Lee AE, Xu Q, Zhang Q, Le AD. Gingiva-Derived Mesenchymal Stem Cells: Potential Application in Tissue Engineering and Regenerative Medicine - A Comprehensive Review. *Front Immunol* 2021; **12**: 667221 [PMID: 33936109 DOI: 10.3389/fimmu.2021.667221]
17 **Martens W**, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrichts I. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. *FASEB J* 2014; **28**: 1634-1643 [PMID: 24352035 DOI: 10.1096/fj.13-243980]
18 **Mattei V**, Martellucci S, Pulcini F, Santilli F, Sorice M, Delle Monache S. Regenerative Potential of DPSCs and Revascularization: Direct, Paracrine or Autocrine Effect? *Stem Cell Rev Rep* 2021; **17**: 1635-1646 [PMID: 33829353 DOI: 10.1007/s12015-021-10162-6]
19 **Junior AL**, Pinheiro CCG, Tanikawa DYS, Ferreira JRM, Amano MT, Bueno DF. Mesenchymal Stem Cells from Human Exfoliated Deciduous Teeth and the Orbicularis Oris Muscle: How Do They Behave When Exposed to a Proinflammatory Stimulus? *Stem Cells Int* 2020; **2020**: 3670412 [PMID: 32184831 DOI: 10.1155/2020/3670412]
20 **Fageeh HN**. Preliminary Evaluation of Proliferation, Wound Healing Properties, Osteogenic and Chondrogenic Potential of Dental Pulp Stem Cells Obtained from Healthy and Periodontitis Affected Teeth. *Cells* 2021; **10** [PMID: 34440887 DOI: 10.3390/cells10082118]
21 **Lin L**, Du L. The role of secreted factors in stem cells-mediated immune regulation. *Cell Immunol* 2018; **326**: 24-32 [PMID: 28778535 DOI: 10.1016/j.cellimm.2017.07.010]
22 **Zhou LL**, Liu W, Wu YM, Sun WL, Dörfer CE, Fawzy El-Sayed KM. Oral Mesenchymal Stem/Progenitor Cells: The Immunomodulatory Masters. *Stem Cells Int* 2020; **2020**: 1327405 [PMID: 32184830 DOI: 10.1155/2020/1327405]
23 **Li W**, Ren G, Huang Y, Su J, Han Y, Li J, Chen X, Cao K, Chen Q, Shou P, Zhang L, Yuan ZR, Roberts AI, Shi S, Le AD, Shi Y. Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell Death Differ* 2012; **19**: 1505-1513 [PMID: 22421969 DOI: 10.1038/cdd.2012.26]

24 **Yan F**, Liu O, Zhang H, Zhou Y, Zhou D, Zhou Z, He Y, Tang Z, Wang S. Human dental pulp stem cells regulate allogeneic NK cells' function *via* induction of anti-inflammatory purinergic signalling in activated NK cells. *Cell Prolif* 2019; **52**: e12595 [PMID: 30953394 DOI: 10.1111/cpr.12595]

25 **Botelho J**, Cavacas MA, Machado V, Mendes JJ. Dental stem cells: recent progresses in tissue engineering and regenerative medicine. *Ann Med* 2017; **49**: 644-651 [PMID: 28649865 DOI: 10.1080/07853890.2017.1347705]

26 **Hu J**, Cao Y, Xie Y, Wang H, Fan Z, Wang J, Zhang C, Wu CT, Wang S. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. *Stem Cell Res Ther* 2016; **7**: 130 [PMID: 27613503 DOI: 10.1186/s13287-016-0362-8]

27 **Ding G**, Liu Y, Wang W, Wei F, Liu D, Fan Z, An Y, Zhang C, Wang S. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. *Stem Cells* 2010; **28**: 1829-1838 [PMID: 20979138 DOI: 10.1002/stem.512]

28 **Xuan K**, Li B, Guo H, Sun W, Kou X, He X, Zhang Y, Sun J, Liu A, Liao L, Liu S, Liu W, Hu C, Shi S, Jin Y. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Sci Transl Med* 2018; **10** [PMID: 30135248 DOI: 10.1126/scitranslmed.aaf3227]

29 **Chen FM**, Gao LN, Tian BM, Zhang XY, Zhang YJ, Dong GY, Lu H, Chu Q, Xu J, Yu Y, Wu RX, Yin Y, Shi S, Jin Y. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. *Stem Cell Res Ther* 2016; **7**: 33 [PMID: 26895633 DOI: 10.1186/s13287-016-0288-1]

30 **Wang SG**, Hsu NC, Wang SM, Wang FN. Successful Treatment of Plaque Psoriasis with Allogeneic Gingival Mesenchymal Stem Cells: A Case Study. *Case Rep Dermatol Med* 2020; **2020**: 4617520 [PMID: 32280547 DOI: 10.1155/2020/4617520]

31 **Horwitz EM**, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A; International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 2005; **7**: 393-395 [PMID: 16236628 DOI: 10.1080/14653240500319234]

32 **Sacchetti B**, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 2007; **131**: 324-336 [PMID: 17956733 DOI: 10.1016/j.cell.2007.08.025]

33 **Chan CKF**, Gulati GS, Sinha R, Tompkins JV, Lopez M, Carter AC, Ransom RC, Reinisch A, Wearda T, Murphy M, Brewer RE, Koepke LS, Marecic O, Manjunath A, Seo EY, Leavitt T, Lu WJ, Nguyen A, Conley SD, Salhotra A, Ambrosi TH, Borrelli MR, Siebel T, Chan K, Schallmoser K, Seita J, Sahoo D, Goodnough H, Bishop J, Gardner M, Majeti R, Wan DC, Goodman S, Weissman IL, Chang HY, Longaker MT. Identification of the Human Skeletal Stem Cell. *Cell* 2018; **175**: 43-56.e21 [PMID: 30241615 DOI: 10.1016/j.cell.2018.07.029]

34 **Viswanathan S**, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, Nolta J, Phinney DG, Sensebe L. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy* 2019; **21**: 1019-1024 [PMID: 31526643 DOI: 10.1016/j.jcyt.2019.08.002]

35 **Nakashima M**, Fukuyama F, Iohara K. Pulp Regenerative Cell Therapy for Mature Molars: A Report of 2 Cases. *J Endod* 2022; **48**: 1334-1340.e1 [PMID: 35940319 DOI: 10.1016/j.joen.2022.07.010]

36 **Meza G**, Urrejola D, Saint Jean N, Inostroza C, López V, Khoury M, Brizuela C. Personalized Cell Therapy for Pulpitis Using Autologous Dental Pulp Stem Cells and Leukocyte Platelet-rich Fibrin: A Case Report. *J Endod* 2019; **45**: 144-149 [PMID: 30711169 DOI: 10.1016/j.joen.2018.11.009]

37 **Nakashima M**, Iohara K, Murakami M, Nakamura H, Sato Y, Ariji Y, Matsushita K. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem Cell Res Ther* 2017; **8**: 61 [PMID: 28279187 DOI: 10.1186/s13287-017-0506-5]

38 **Shiehzadeh V**, Aghmasheh F, Shiehzadeh F, Joulae M, Kosarieh E. Healing of large periapical lesions following delivery of dental stem cells with an injectable scaffold: new method and three case reports. *Indian J Dent Res* 2014; **25**: 248-253 [PMID: 24992862 DOI: 10.4103/0970-9290.135937]

39 **Prasad MGS**, Ramakrishna J, Babu DN. Allogeneic stem cells derived from human exfoliated deciduous teeth (SHED) for the management of periapical lesions in permanent teeth: Two case reports of a novel biologic alternative treatment. *J Dent Res Dent Clin Dent Prospects* 2017; **11**: 117-122 [PMID: 28748053 DOI: 10.15171/joddd.2017.021]

40 **Ghana Shyam Prasad M**, Juvva R, Babu Duvvi N. Towards a New Era in the Management of Large Periapical Lesion in Permanent Tooth Using Stemcells: A 2-Year Clinical Application Report. *J Dent (Shiraz)* 2019; **20**: 137-140 [PMID: 31214643 DOI: 10.30476/dentjods.2019.44925]

41 **Ferrarotti F**, Romano F, Gamba MN, Quirico A, Giraudi M, Audagna M, Aimetti M. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial. *J Clin Periodontol* 2018; **45**: 841-850 [PMID: 29779220 DOI: 10.1111/jcpe.12931]

42 **Sánchez N**, Fierravanti L, Núñez J, Vignoletti F, González-Zamora M, Santamaría S, Suárez-Sancho S, Fernández-Santos ME, Figuero E, Herrera D, García-Sanz JA, Sanz M. Periodontal regeneration using a xenogeneic bone substitute seeded with autologous periodontal ligament-derived mesenchymal stem cells: A 12-month quasi-randomized controlled pilot clinical trial. *J Clin Periodontol* 2020; **47**: 1391-1402 [PMID: 32946590 DOI: 10.1111/jcpe.13368]

43 **Feng F**, Akiyama K, Liu Y, Yamaza T, Wang TM, Chen JH, Wang BB, Huang GT, Wang S, Shi S. Utility of PDL progenitors for *in vivo* tissue regeneration: a report of 3 cases. *Oral Dis* 2010; **16**: 20-28 [PMID: 20355278 DOI: 10.1111/j.1601-0825.2009.01593.x]

44 **Iwata T**, Yamato M, Washio K, Yoshida T, Tsumanuma Y, Yamada A, Onizuka S, Izumi Y, Ando T, Okano T, Ishikawa I. Periodontal regeneration with autologous periodontal ligament-derived cell sheets - A safety and efficacy study in ten patients. *Regen Ther* 2018; **9**: 38-44 [PMID: 30525074 DOI: 10.1016/j.reth.2018.07.002]

45 **Kl V**, Ryana H, Dalvi PJ. Autologous periodontal stem cell assistance in periodontal regeneration technique (SAI-PRT) in the treatment of periodontal intrabony defects: A case report with one-year follow-up. *J Dent Res Dent Clin Dent Prospects* 2017; **11**: 123-126 [PMID: 28748054 DOI: 10.15171/joddd.2017.022]

46 **Aimetti M**, Ferrarotti F, Gamba MN, Giraudi M, Romano F. Regenerative Treatment of Periodontal Intrabony Defects

Using Autologous Dental Pulp Stem Cells: A 1-Year Follow-Up Case Series. *Int J Periodontics Restorative Dent* 2018; **38**: 51-58 [PMID: 29240205 DOI: 10.11607/prd.3425]

47 **Aimetti M**, Ferrarotti F, Cricenti L, Mariani GM, Romano F. Autologous dental pulp stem cells in periodontal regeneration: a case report. *Int J Periodontics Restorative Dent* 2014; **34** Suppl 3: s27-s33 [PMID: 24956088 DOI: 10.11607/prd.1635]

48 **Hernández-Monjaraz B**, Santiago-Osorio E, Ledesma-Martínez E, Alcauter-Zavala A, Mendoza-Núñez VM. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report. *J Int Med Res* 2018; **46**: 2983-2993 [PMID: 29911458 DOI: 10.1177/0300060518773244]

49 **Aimetti M**, Ferrarotti F, Mariani GM, Cricenti L, Romano F. Use of Dental Pulp Stem Cells/Collagen Sponge Biocomplex in the Treatment of Non-Contained Intrabony Defects: A Case Series. *Clin Adv Periodontics* 2015; **5**: 104-109 [PMID: 32689724 DOI: 10.1902/cap.2013.130047]

50 **He H**, Yu J, Cao J, E L, Wang D, Zhang H, Liu H. Biocompatibility and Osteogenic Capacity of Periodontal Ligament Stem Cells on nHAC/PLA and HA/TCP Scaffolds. *J Biomater Sci Polym Ed* 2011; **22**: 179-194 [PMID: 20557694 DOI: 10.1163/092050609X12587018007767]

51 **Xia L**, Zhang Z, Chen L, Zhang W, Zeng D, Zhang X, Chang J, Jiang X. Proliferation and osteogenic differentiation of human periodontal ligament cells on akermanite and β-TCP bioceramics. *Eur Cell Mater* 2011; **22**: 68-82; discussion 83 [PMID: 21761393 DOI: 10.22203/ecm.v022a06]

52 **Yu BH**, Zhou Q, Wang ZL. Periodontal ligament versus bone marrow mesenchymal stem cells in combination with Bio-Oss scaffolds for ectopic and in situ bone formation: A comparative study in the rat. *J Biomater Appl* 2014; **29**: 243-253 [PMID: 24487130 DOI: 10.1177/0885328214521846]

53 **Fu X**, Jin L, Ma P, Fan Z, Wang S. Allogeneic stem cells from deciduous teeth in treatment for periodontitis in miniature swine. *J Periodontol* 2014; **85**: 845-851 [PMID: 24001042 DOI: 10.1902/jop.2013.130254]

54 **Maroo S**, Murthy KR. Treatment of periodontal intrabony defects using β-TCP alone or in combination with rhPDGF-BB: a randomized controlled clinical and radiographic study. *Int J Periodontics Restorative Dent* 2014; **34**: 841-847 [PMID: 25411740 DOI: 10.11607/prd.2030]

55 **Zafiropoulos GK**, Hoffmann O, Kasaj A, Willershausen B, Weiss O, Van Dyke TE. Treatment of Intrabony Defects Using Guided Tissue Regeneration and Autogenous Spongiosa Alone or Combined With Hydroxyapatite/β-Tricalcium Phosphate Bone Substitute or Bovine-Derived Xenograft. *J Periodontol* 2007; **78**: 2216-2225 [PMID: 29539135 DOI: 10.1902/jop.2007.070146]

56 **Camelo M**, Nevins ML, Schenk RK, Simion M, Rasperini G, Lynch SE, Nevins M. Clinical, radiographic, and histologic evaluation of human periodontal defects treated with Bio-Oss and Bio-Gide. *Int J Periodontics Restorative Dent* 1998; **18**: 321-331 [PMID: 12693419]

57 **Barbier L**, Ramos E, Mendiola J, Rodriguez O, Santamaria G, Santamaria J, Arteagoitia I. Autologous dental pulp mesenchymal stem cells for inferior third molar post-extraction socket healing: A split-mouth randomised clinical trial. *Med Oral Patol Oral Cir Bucal* 2018; **23**: e469-e477 [PMID: 29924768 DOI: 10.4317/medoral.22466]

58 **d'Aquino R**, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* 2009; **18**: 75-83 [PMID: 19908196 DOI: 10.22203/ecm.v018a07]

59 **Manimaran K**, Sankaranarayanan S, Ravi VR, Elangovan S, Chandramohan M, Perumal SM. Treatment of osteoradionecrosis of mandible with bone marrow concentrate and with dental pulp stem cells. *Ann Maxillofac Surg* 2014; **4**: 189-192 [PMID: 25593871 DOI: 10.4103/2231-0746.147130]

60 **Manimaran K**, Sharma R, Sankaranarayanan S, Perumal SM. Regeneration of mandibular ameloblastoma defect with the help of autologous dental pulp stem cells and buccal pad of fat stromal vascular fraction. *Ann Maxillofac Surg* 2016; **6**: 97-100 [PMID: 27563616 DOI: 10.4103/2231-0746.186128]

61 **Brunelli G**, Motroni A, Graziano A, D'Aquino R, Zollino I, Carinci F. Sinus lift tissue engineering using autologous pulp micro-grafts: A case report of bone density evaluation. *J Indian Soc Periodontol* 2013; **17**: 644-647 [PMID: 24174760 DOI: 10.4103/0972-124X.119284]

62 **Cubuk S**, Oduncuoglu BF, Alaaddinoglu EE. The effect of dental pulp stem cells and L-PRF when placed into the extraction sockets of impacted mandibular third molars on the periodontal status of adjacent second molars: a split-mouth, randomized, controlled clinical trial. *Oral Maxillofac Surg* 2023; **27**: 59-68 [PMID: 35141806 DOI: 10.1007/s10006-022-01045-2]

63 **Tanikawa DYS**, Pinheiro CCG, Almeida MCA, Oliveira CRGCM, Coudry RA, Rocha DL, Bueno DF. Deciduous Dental Pulp Stem Cells for Maxillary Alveolar Reconstruction in Cleft Lip and Palate Patients. *Stem Cells Int* 2020; **2020**: 6234167 [PMID: 32256610 DOI: 10.1155/2020/6234167]

64 **Koga S**, Horiguchi Y. Efficacy of a cultured conditioned medium of exfoliated deciduous dental pulp stem cells in erectile dysfunction patients. *J Cell Mol Med* 2022; **26**: 195-201 [PMID: 34845823 DOI: 10.1111/jcmm.17072]

65 **da Silva JM**, Araldi RP, Colozza-Gama GA, Pagani E, Sid A, Valverde CW, Kerkis I. Human Immature Dental Pulp Stem Cells Did Not Graft into a Preexisting Human Lung Adenocarcinoma. *Case Rep Oncol* 2022; **15**: 413-422 [PMID: 35702561 DOI: 10.1159/000523896]

66 Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. *Mov Disord* 1996; **11**: 136-142 [PMID: 8684382 DOI: 10.1002/mds.870110204]

67 **Suda S**, Nito C, Ihara M, Iguchi Y, Urabe T, Matsumaru Y, Sakai N, Kimura K; J- REPAIR trial group. Randomised placebo-controlled multicentre trial to evaluate the efficacy and safety of JTR-161, allogeneic human dental pulp stem cells, in patients with Acute Ischaemic stRoke (J-REPAIR). *BMJ Open* 2022; **12**: e054269 [PMID: 35613802 DOI: 10.1136/bmjopen-2021-054269]

68 **Nagpal A**, Kremer KL, Hamilton-Bruce MA, Kaidonis X, Milton AG, Levi C, Shi S, Carey L, Hillier S, Rose M, Zacest A, Takhar P, Koblar SA. TOOTH (The Open study Of dental pulp stem cell Therapy in Humans): Study protocol for evaluating safety and feasibility of autologous human adult dental pulp stem cell therapy in patients with chronic disability after stroke. *Int J Stroke* 2016; **11**: 575-585 [PMID: 27030504 DOI: 10.1177/1747493016641111]

69 **Ye Q**, Wang H, Xia X, Zhou C, Liu Z, Xia ZE, Zhang Z, Zhao Y, Yehenala J, Wang S, Zhou G, Hu K, Wu B, Wu CT, He Y. Safety and efficacy assessment of allogeneic human dental pulp stem cells to treat patients with severe COVID-19: structured summary of a study protocol for a randomized controlled trial (Phase I / II). *Trials* 2020; **21**: 520 [PMID: 32532356 DOI: 10.1186/s13063-020-04380-5]

70 **Pinheiro CCG**, Leyendecker Junior A, Tanikawa DYS, Ferreira JRM, Jarrahy R, Bueno DF. Is There a Noninvasive Source of MSCs Isolated with GMP Methods with Better Osteogenic Potential? *Stem Cells Int* 2019; **2019**: 7951696 [PMID: 31781247 DOI: 10.1155/2019/7951696]

71 **Wenceslau CV**, de Souza DM, Mambelli-Lisboa NC, Ynoue LH, Araldi RP, da Silva JM, Pagani E, Haddad MS, Kerkis I. Restoration of BDNF, DARPP32, and D2R Expression Following Intravenous Infusion of Human Immature Dental Pulp Stem Cells in Huntington's Disease 3-NP Rat Model. *Cells* 2022; **11** [PMID: 35626701 DOI: 10.3390/cells11101664]

72 **Volarevic V**, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, Armstrong L, Djonov V, Lako M, Stojkovic M. Ethical and Safety Issues of Stem Cell-Based Therapy. *Int J Med Sci* 2018; **15**: 36-45 [PMID: 29333086 DOI: 10.7150/ijms.21666]

73 **Breitbach M**, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JW, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SE, Fleischmann BK. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 2007; **110**: 1362-1369 [PMID: 17483296 DOI: 10.1182/blood-2006-12-063412]

74 **Yoon YS**, Park JS, Tkebuchava T, Luedeman C, Losordo DW. Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation* 2004; **109**: 3154-3157 [PMID: 15197139 DOI: 10.1161/01.Cir.0000134696.08436.65]

75 **Hmadcha A**, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic Potential of Mesenchymal Stem Cells for Cancer Therapy. *Front Bioeng Biotechnol* 2020; **8**: 43 [PMID: 32117924 DOI: 10.3389/fbioe.2020.00043]

76 **Lan T**, Luo M, Wei X. Mesenchymal stem/stromal cells in cancer therapy. *J Hematol Oncol* 2021; **14**: 195 [PMID: 34789315 DOI: 10.1186/s13045-021-01208-w]

77 **Merckx G**, Lo Monaco M, Lambrichts I, Himmelreich U, Bronckaers A, Wolfs E. Safety and Homing of Human Dental Pulp Stromal Cells in Head and Neck Cancer. *Stem Cell Rev Rep* 2021; **17**: 1619-1634 [PMID: 33822326 DOI: 10.1007/s12015-021-10159-1]

78 **Whiteside TL**. Exosome and mesenchymal stem cell cross-talk in the tumor microenvironment. *Semin Immunol* 2018; **35**: 69-79 [PMID: 29289420 DOI: 10.1016/j.smim.2017.12.003]

79 **Ridge SM**, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. *Mol Cancer* 2017; **16**: 31 [PMID: 28148268 DOI: 10.1186/s12943-017-0597-8]

80 **Doğan A**, Demirci S, Apdik H, Apdik EA, Şahin F. Dental pulp stem cells (DPSCs) increase prostate cancer cell proliferation and migration under in vitro conditions. *Tissue Cell* 2017; **49**: 711-718 [PMID: 29054337 DOI: 10.1016/j.tice.2017.10.003]

81 **Raj AT**, Kheur S, Khurshid Z, Sayed ME, Mugri MH, Almasri MA, Al-Ahmari MM, Patil VR, Bhandi S, Testarelli L, Patil S. The Growth Factors and Cytokines of Dental Pulp Mesenchymal Stem Cell Secretome May Potentially Aid in Oral Cancer Proliferation. *Molecules* 2021; **26** [PMID: 34577154 DOI: 10.3390/molecules26185683]

82 **Raj AT**, Kheur S, Bhonde R, Mani VR, Baeshen HA, Patil S. Assessing the effect of human dental pulp mesenchymal stem cell secretome on human oral, breast, and melanoma cancer cell lines. *Saudi J Biol Sci* 2021; **28**: 6556-6567 [PMID: 34764771 DOI: 10.1016/j.sjbs.2021.07.029]

83 **Sun B**, Roh KH, Park JR, Lee SR, Park SB, Jung JW, Kang SK, Lee YS, Kang KS. Therapeutic potential of mesenchymal stromal cells in a mouse breast cancer metastasis model. *Cytotherapy* 2009; **11**: 289-298, 1 p following 298 [PMID: 19308770 DOI: 10.1080/14653240902807026]

84 **Khakoo AY**, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, Nguyen AT, Malide D, Combs CA, Hall G, Zhang J, Raffeld M, Rogers TB, Stetler-Stevenson W, Frank JA, Reitz M, Finkel T. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006; **203**: 1235-1247 [PMID: 16636132 DOI: 10.1084/jem.20051921]

85 **Qiao L**, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, Ye L, Zhang X. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. *Cell Res* 2008; **18**: 500-507 [PMID: 18364678 DOI: 10.1038/cr.2008.40]

86 **Nikkhah E**, Kalalinia F, Asgharian Rezaee M, Tayarani-Najaran Z. Suppressive effects of dental pulp stem cells and its conditioned medium on development and migration of colorectal cancer cells through MAPKinase pathways. *Iran J Basic Med Sci* 2021; **24**: 1292-1300 [PMID: 35083017 DOI: 10.22038/ijbms.2021.58273.12946]

87 **Neuhaus KW**. Teeth: malignant neoplasms in the dental pulp? *Lancet Oncol* 2007; **8**: 75-78 [PMID: 17196513 DOI: 10.1016/s1470-2045(06)71013-0]

88 **Shen WC**, Lai YC, Li LH, Liao K, Lai HC, Kao SY, Wang J, Chuong CM, Hung SC. Methylation and PTEN activation in dental pulp mesenchymal stem cells promotes osteogenesis and reduces oncogenesis. *Nat Commun* 2019; **10**: 2226 [PMID: 31110221 DOI: 10.1038/s41467-019-10197-x]

89 **Álvarez-Garcia V**, Tawil Y, Wise HM, Leslie NR. Mechanisms of PTEN loss in cancer: It's all about diversity. *Semin Cancer Biol* 2019; **59**: 66-79 [PMID: 30738865 DOI: 10.1016/j.semcancer.2019.02.001]

90 **Li X**, Makarov SS. An essential role of NF-kappaB in the "tumor-like" phenotype of arthritic synoviocytes. *Proc Natl Acad Sci U S A* 2006; **103**: 17432-17437 [PMID: 17088540 DOI: 10.1073/pnas.0607939103]

91 **Gilbert L**, He X, Farmer P, Boden S, Kozlowski M, Rubin J, Nanes MS. Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. *Endocrinology* 2000; **141**: 3956-3964 [PMID: 11089525 DOI: 10.1210/endo.141.11.7739]

92 **Alongi DJ**, Yamaza T, Song Y, Fouad AF, Romberg EE, Shi S, Tuan RS, Huang GT. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. *Regen Med* 2010; **5**: 617-631 [PMID: 20465527 DOI: 10.2217/rme.10.30]

93 **Wang Z**, Pan J, Wright JT, Bencharit S, Zhang S, Everett ET, Teixeira FB, Preisser JS. Putative stem cells in human dental pulp with irreversible pulpitis: an exploratory study. *J Endod* 2010; **36**: 820-825 [PMID: 20416426 DOI: 10.1016/j.joen.2010.02.003]

94 **Pereira LO**, Rubini MR, Silva JR, Oliveira DM, Silva IC, Poças-Fonseca MJ, Azevedo RB. Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. *Int Endod J* 2012; **45**: 1080-1090 [PMID: 22747502 DOI: 10.1111/j.1365-2591.2012.02068.x]

95 **Attar A**, Eslaminejad MB, Tavangar MS, Karamzadeh R, Dehghani-Nazhvani A, Ghahramani Y, Malekmohammadi F, Hosseini SM. Dental pulp polyps contain stem cells comparable to the normal dental pulps. *J Clin Exp Dent* 2014; **6**: e53-e59 [PMID: 24596636 DOI: 10.4317/jced.51305]

96 **Park YT**, Lee SM, Kou X, Karabucak B. The Role of Interleukin 6 in Osteogenic and Neurogenic Differentiation Potentials of Dental Pulp Stem Cells. *J Endod* 2019; **45**: 1342-1348 [PMID: 31540748 DOI: 10.1016/j.joen.2019.08.002]

97 **Nie SC**, Yang K, Luan NN, Lian XL, Dai XH, Liang SX, Yan YB. Unveiling the Differences in Biological Properties of Dental Pulp Stem Cells from Normal and Inflamed Pulp: A Comprehensive Comparative Study. *Med Sci Monit* 2022; **28**: e934511 [PMID: 35301274 DOI: 10.12659/MSM.934511]

98 **Tomasello L**, Mauceri R, Coppola A, Pitrone M, Pizzo G, Campisi G, Pizzolanti G, Giordano C. Mesenchymal stem cells derived from inflamed dental pulpal and gingival tissue: a potential application for bone formation. *Stem Cell Res Ther* 2017; **8**: 179 [PMID: 28764802 DOI: 10.1186/s13287-017-0633-z]

99 **Zaky SH**, Shehabeldin M, Ray H, Sfeir C. The role of inflammation modulation in dental pulp regeneration. *Eur Cell Mater* 2021; **41**: 184-193 [PMID: 33583014 DOI: 10.22203/eCM.v041a13]

100 **Cacci E**, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L. In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* 2008; **56**: 412-425 [PMID: 18186084 DOI: 10.1002/glia.20616]

101 **Kato M**, Tsunekawa S, Nakamura N, Miura-Yura E, Yamada Y, Hayashi Y, Nakai-Shimoda H, Asano S, Hayami T, Motegi M, Asano-Hayami E, Sasajima S, Morishita Y, Himeno T, Kondo M, Kato Y, Izumoto-Akita T, Yamamoto A, Naruse K, Nakamura J, Kamiya H. Secreted Factors from Stem Cells of Human Exfoliated Deciduous Teeth Directly Activate Endothelial Cells to Promote All Processes of Angiogenesis. *Cells* 2020; **9** [PMID: 33142678 DOI: 10.3390/cells9112385]

102 **Hochuli AHD**, Senegaglia AC, Selenko AH, Fracaro L, Brofman PRS. Dental Pulp from Human Exfoliated Deciduous Teeth-derived Stromal Cells Demonstrated Neuronal Potential: *In vivo* and In Vitro Studies. *Curr Stem Cell Res Ther* 2021; **16**: 495-506 [PMID: 33588741 DOI: 10.2174/1574888X16666210215160402]

103 **Hiraki T**, Kunimatsu R, Nakajima K, Abe T, Yamada S, Rikitake K, Tanimoto K. Stem cell-derived conditioned media from human exfoliated deciduous teeth promote bone regeneration. *Oral Dis* 2020; **26**: 381-390 [PMID: 31808229 DOI: 10.1111/odi.13244]

104 **Lin H**, Chen H, Zhao X, Chen Z, Zhang P, Tian Y, Wang Y, Ding T, Wang L, Shen Y. Advances in mesenchymal stem cell conditioned medium-mediated periodontal tissue regeneration. *J Transl Med* 2021; **19**: 456 [PMID: 34736500 DOI: 10.1186/s12967-021-03125-5]

105 **Makino E**, Nakamura N, Miyabe M, Ito M, Kanada S, Hata M, Saiki T, Sango K, Kamiya H, Nakamura J, Miyazawa K, Goto S, Matsubara T, Naruse K. Conditioned media from dental pulp stem cells improved diabetic polyneuropathy through anti-inflammatory, neuroprotective and angiogenic actions: Cell-free regenerative medicine for diabetic polyneuropathy. *J Diabetes Investig* 2019; **10**: 1199-1208 [PMID: 30892819 DOI: 10.1111/jdi.13045]

106 **de Cara SPHM**, Origassa CST, de Sá Silva F, Moreira MSNA, de Almeida DC, Pedroni ACF, Carvalho GL, Cury DP, Câmara NOS, Marques MM. Angiogenic properties of dental pulp stem cells conditioned medium on endothelial cells in vitro and in rodent orthotopic dental pulp regeneration. *Heliyon* 2019; **5**: e01560 [PMID: 31183428 DOI: 10.1016/j.heliyon.2019.e01560]

107 **Ueda T**, Ito T, Inden M, Kurita H, Yamamoto A, Hozumi I. Stem Cells From Human Exfoliated Deciduous Teeth-Conditioned Medium (SHED-CM) is a Promising Treatment for Amyotrophic Lateral Sclerosis. *Front Pharmacol* 2022; **13**: 805379 [PMID: 35185565 DOI: 10.3389/fphar.2022.805379]

108 **Sugimura-Wakayama Y**, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, Hibi H. Peripheral Nerve Regeneration by Secretomes of Stem Cells from Human Exfoliated Deciduous Teeth. *Stem Cells Dev* 2015; **24**: 2687-2699 [PMID: 26154068 DOI: 10.1089/scd.2015.0104]

109 **Tsuruta T**, Sakai K, Watanabe J, Katagiri W, Hibi H. Dental pulp-derived stem cell conditioned medium to regenerate peripheral nerves in a novel animal model of dysphagia. *PLoS One* 2018; **13**: e0208938 [PMID: 30533035 DOI: 10.1371/journal.pone.0208938]

110 **Hirata M**, Ishigami M, Matsushita Y, Ito T, Hattori H, Hibi H, Goto H, Ueda M, Yamamoto A. Multifaceted Therapeutic Benefits of Factors Derived From Dental Pulp Stem Cells for Mouse Liver Fibrosis. *Stem Cells Transl Med* 2016; **5**: 1416-1424 [PMID: 27280796 DOI: 10.5966/sctm.2015-0353]

111 **Matsushita Y**, Ishigami M, Matsubara K, Kondo M, Wakayama H, Goto H, Ueda M, Yamamoto A. Multifaceted therapeutic benefits of factors derived from stem cells from human exfoliated deciduous teeth for acute liver failure in rats. *J Tissue Eng Regen Med* 2017; **11**: 1888-1896 [PMID: 28586545 DOI: 10.1002/term.2086]

112 **Wakayama H**, Hashimoto N, Matsushita Y, Matsubara K, Yamamoto N, Hasegawa Y, Ueda M, Yamamoto A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating acute lung injury in mice. *Cytotherapy* 2015; **17**: 1119-1129 [PMID: 26031744 DOI: 10.1016/j.jcyt.2015.04.009]

113 **Ogasawara N**, Kano F, Hashimoto N, Mori H, Liu Y, Xia L, Sakamaki T, Hibi H, Iwamoto T, Tanaka E, Yamamoto A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental temporomandibular joint osteoarthritis. *Osteoarthritis Cartilage* 2020; **28**: 831-841 [PMID: 32272195 DOI: 10.1016/j.joca.2020.03.010]

114 **Matsumura-Kawashima M**, Ogata K, Moriyama M, Murakami Y, Kawado T, Nakamura S. Secreted factors from dental pulp stem cells improve Sjögren's syndrome *via* regulatory T cell-mediated immunosuppression. *Stem Cell Res Ther* 2021; **12**: 182 [PMID: 33726818 DOI: 10.1186/s13287-021-02236-6]

115 **Ishikawa J**, Takahashi N, Matsumoto T, Yoshioka Y, Yamamoto N, Nishikawa M, Hibi H, Ishigro N, Ueda M, Furukawa K, Yamamoto A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis. *Bone* 2016; **83**: 210-219 [PMID: 26603475 DOI: 10.1016/j.bone.2015.11.012]

116 **Muto H**, Ito T, Tanaka T, Yokoyama S, Yamamoto K, Imai N, Ishizu Y, Maeda K, Honda T, Ishikawa T, Kato A,

Ohshiro T, Kano F, Yamamoto A, Sakai K, Hibi H, Ishigami M, Fujishiro M. Conditioned medium from stem cells derived from human exfoliated deciduous teeth ameliorates NASH *via* the Gut-Liver axis. *Sci Rep* 2021; **11**: 18778 [PMID: 34548598 DOI: 10.1038/s41598-021-98254-8]

117 **Imanishi Y**, Hata M, Matsukawa R, Aoyagi A, Omi M, Mizutani M, Naruse K, Ozawa S, Honda M, Matsubara T, Takebe J. Efficacy of extracellular vesicles from dental pulp stem cells for bone regeneration in rat calvarial bone defects. *Inflamm Regen* 2021; **41**: 12 [PMID: 33853679 DOI: 10.1186/s41232-021-00163-w]

118 **Lee AE**, Choi JG, Shi SH, He P, Zhang QZ, Le AD. DPSC-Derived Extracellular Vesicles Promote Rat Jawbone Regeneration. *J Dent Res* 2023; **102**: 313-321 [PMID: 36348514 DOI: 10.1177/00220345221133716]

119 **Zhou Z**, Zheng J, Lin D, Xu R, Chen Y, Hu X. Exosomes derived from dental pulp stem cells accelerate cutaneous wound healing by enhancing angiogenesis *via* the Cdc42/p38 MAPK pathway. *Int J Mol Med* 2022; **50** [PMID: 36321793 DOI: 10.3892/ijmm.2022.5199]

120 **Merckx G**, Hosseinkhani B, Kuypers S, Deville S, Irobi J, Nelissen I, Michiels L, Lambrichts I, Bronckaers A. Angiogenic Effects of Human Dental Pulp and Bone Marrow-Derived Mesenchymal Stromal Cells and their Extracellular Vesicles. *Cells* 2020; **9** [PMID: 32012900 DOI: 10.3390/cells9020312]

121 **Li S**, Luo L, He Y, Li R, Xiang Y, Xing Z, Li Y, Albashari AA, Liao X, Zhang K, Gao L, Ye Q. Dental pulp stem cell-derived exosomes alleviate cerebral ischaemia-reperfusion injury through suppressing inflammatory response. *Cell Prolif* 2021; **54**: e13093 [PMID: 34231932 DOI: 10.1111/cpr.13093]

122 **Abdelwahab S**, Elsebay SAG, Fouli Gaber M, Abdel-Hafez SMN. Comparative study between bone marrow mesenchymal stem cell and their conditioned medium in the treatment of rat model of Parkinsonism. *J Cell Physiol* 2021; **236**: 440-457 [PMID: 32557610 DOI: 10.1002/jcp.29872]

123 **Hashemi SM**, Hassan ZM, Hossein-Khannazer N, Pourfathollah AA, Soudi S. Investigating the route of administration and efficacy of adipose tissue-derived mesenchymal stem cells and conditioned medium in type 1 diabetic mice. *Inflammopharmacology* 2020; **28**: 585-601 [PMID: 31741175 DOI: 10.1007/s10787-019-00661-x]

124 **Kovach TK**, Dighe AS, Lobo PI, Cui Q. Interactions between MSCs and immune cells: implications for bone healing. *J Immunol Res* 2015; **2015**: 752510 [PMID: 26000315 DOI: 10.1155/2015/752510]

125 **Vandana KL**, Desai R, Dalvi PJ. Autologous Stem Cell Application in Periodontal Regeneration Technique (SAI-PRT) Using PDLSCs Directly From an Extracted Tooth···An Insight. *Int J Stem Cells* 2015; **8**: 235-237 [PMID: 26634072 DOI: 10.15283/ijsc.2015.8.2.235]

126 **Monti M**, Graziano A, Rizzo S, Perotti C, Del Fante C, d'Aquino R, Redi CA, Rodriguez Y Baena R. In Vitro and *In vivo* Differentiation of Progenitor Stem Cells Obtained After Mechanical Digestion of Human Dental Pulp. *J Cell Physiol* 2017; **232**: 548-555 [PMID: 27277190 DOI: 10.1002/jcp.25452]

127 **Vazin T**, Freed WJ. Human embryonic stem cells: derivation, culture, and differentiation: a review. *Restor Neurol Neurosci* 2010; **28**: 589-603 [PMID: 20714081 DOI: 10.3233/RNN-2010-0543]

128 **Yamanaka S**. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. *Cell Stem Cell* 2020; **27**: 523-531 [PMID: 33007237 DOI: 10.1016/j.stem.2020.09.014]

129 **Ohnuki M**, Takahashi K. Present and future challenges of induced pluripotent stem cells. *Philos Trans R Soc Lond B Biol Sci* 2015; **370**: 20140367 [PMID: 26416678 DOI: 10.1098/rstb.2014.0367]

130 **Anisimov SV**, Morizane A, Correia AS. Risks and mechanisms of oncological disease following stem cell transplantation. *Stem Cell Rev Rep* 2010; **6**: 411-424 [PMID: 20300888 DOI: 10.1007/s12015-010-9134-5]

131 **Wang H**, Gong P, Li J, Fu Y, Zhou Z, Liu L. Role of CD133 in human embryonic stem cell proliferation and teratoma formation. *Stem Cell Res Ther* 2020; **11**: 208 [PMID: 32460847 DOI: 10.1186/s13287-020-01729-0]

132 **Han L**, He H, Yang Y, Meng Q, Ye F, Chen G, Zhang J. Distinctive Clinical and Pathologic Features of Immature Teratomas Arising from Induced Pluripotent Stem Cell-Derived Beta Cell Injection in a Diabetes Patient. *Stem Cells Dev* 2022; **31**: 97-101 [PMID: 35018826 DOI: 10.1089/scd.2021.0255]

133 **Lee MO**, Moon SH, Jeong HC, Yi JY, Lee TH, Shim SH, Rhee YH, Lee SH, Oh SJ, Lee MY, Han MJ, Cho YS, Chung HM, Kim KS, Cha HJ. Inhibition of pluripotent stem cell-derived teratoma formation by small molecules. *Proc Natl Acad Sci U S A* 2013; **110**: E3281-E3290 [PMID: 23918355 DOI: 10.1073/pnas.1303669110]

134 **Okita K**, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, Hong H, Nakagawa M, Tanabe K, Tezuka K, Shibata T, Kunisada T, Takahashi M, Takahashi J, Saji H, Yamanaka S. A more efficient method to generate integration-free human iPS cells. *Nat Methods* 2011; **8**: 409-412 [PMID: 21460823 DOI: 10.1038/nmeth.1591]

135 **Nakagawa M**, Taniguchi Y, Senda S, Takizawa N, Ichisaka T, Asano K, Morizane A, Doi D, Takahashi J, Nishizawa M, Yoshida Y, Toyoda T, Osafune K, Sekiguchi K, Yamanaka S. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. *Sci Rep* 2014; **4**: 3594 [PMID: 24399248 DOI: 10.1038/srep03594]

136 **Miyazaki T**, Futaki S, Suemori H, Taniguchi Y, Yamada M, Kawasaki M, Hayashi M, Kumagai H, Nakatsuji N, Sekiguchi K, Kawase E. Laminin E8 fragments support efficient adhesion and expansion of dissociated human pluripotent stem cells. *Nat Commun* 2012; **3**: 1236 [PMID: 23212365 DOI: 10.1038/ncomms2231]

137 **Suman S**, Domingues A, Ratajczak J, Ratajczak MZ. Potential Clinical Applications of Stem Cells in Regenerative Medicine. *Adv Exp Med Biol* 2019; **1201**: 1-22 [PMID: 31898779 DOI: 10.1007/978-3-030-31206-0_1]

138 **Hilfiker A**, Kasper C, Hass R, Haverich A. Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation? *Langenbecks Arch Surg* 2011; **396**: 489-497 [PMID: 21373941 DOI: 10.1007/s00423-011-0762-2]

139 **Sharpe PT**. Dental mesenchymal stem cells. *Development* 2016; **143**: 2273-2280 [PMID: 27381225 DOI: 10.1242/dev.134189]

140 **Bakopoulou A**, Apatzidou D, Aggelidou E, Gousopoulou E, Leyhausen G, Volk J, Kritis A, Koidis P, Geurtsen W. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects "stemness" properties. *Stem Cell Res Ther* 2017; **8**: 247 [PMID: 29096714 DOI: 10.1186/s13287-017-0705-0]

141 **Niehage C**, Karbanová J, Steenblock C, Corbeil D, Hoflack B. Cell Surface Proteome of Dental Pulp Stem Cells Identified by Label-Free Mass Spectrometry. *PLoS One* 2016; **11**: e0159824 [PMID: 27490675 DOI:

10.1371/journal.pone.0159824]
142 **Shoi K**, Aoki K, Ohya K, Takagi Y, Shimokawa H. Characterization of pulp and follicle stem cells from impacted supernumerary maxillary incisors. *Pediatr Dent* 2014; **36**: 79-84 [PMID: 24960375]
143 **Hu L**, Zhao B, Gao Z, Xu J, Fan Z, Zhang C, Wang J, Wang S. Regeneration characteristics of different dental derived stem cell sheets. *J Oral Rehabil* 2020; **47** Suppl 1: 66-72 [PMID: 31211857 DOI: 10.1111/joor.12839]
144 **Li D**, Zou XY, El-Ayachi I, Romero LO, Yu Z, Iglesias-Linares A, Cordero-Morales JF, Huang GT. Human Dental Pulp Stem Cells and Gingival Mesenchymal Stem Cells Display Action Potential Capacity In Vitro after Neuronogenic Differentiation. *Stem Cell Rev Rep* 2019; **15**: 67-81 [PMID: 30324358 DOI: 10.1007/s12015-018-9854-5]
145 **Sabbagh J**, Ghassibe-Sabbagh M, Fayyad-Kazan M, Al-Nemer F, Fahed JC, Berberi A, Badran B. Differences in osteogenic and odontogenic differentiation potential of DPSCs and SHED. *J Dent* 2020; **101**: 103413 [PMID: 32585262 DOI: 10.1016/j.jdent.2020.103413]
146 **Kumar A**, Kumar V, Rattan V, Jha V, Pal A, Bhattacharyya S. Molecular spectrum of secretome regulates the relative hepatogenic potential of mesenchymal stem cells from bone marrow and dental tissue. *Sci Rep* 2017; **7**: 15015 [PMID: 29118330 DOI: 10.1038/s41598-017-14358-0]
147 **Trubiani O**, Pizzicannella J, Caputi S, Marchisio M, Mazzon E, Paganelli R, Paganelli A, Diomede F. Periodontal Ligament Stem Cells: Current Knowledge and Future Perspectives. *Stem Cells Dev* 2019; **28**: 995-1003 [PMID: 31017047 DOI: 10.1089/scd.2019.0025]
148 **Seo BM**, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; **364**: 149-155 [PMID: 15246727 DOI: 10.1016/s0140-6736(04)16627-0]
149 **Kawanabe N**, Murata S, Murakami K, Ishihara Y, Hayano S, Kurosaka H, Kamioka H, Takano-Yamamoto T, Yamashiro T. Isolation of multipotent stem cells in human periodontal ligament using stage-specific embryonic antigen-4. *Differentiation* 2010; **79**: 74-83 [PMID: 19945209 DOI: 10.1016/j.diff.2009.10.005]
150 **Kang J**, Fan W, Deng Q, He H, Huang F. Stem Cells from the Apical Papilla: A Promising Source for Stem Cell-Based Therapy. *Biomed Res Int* 2019; **2019**: 6104738 [PMID: 30834270 DOI: 10.1155/2019/6104738]
151 **Bakopoulou A**, Leyhausen G, Volk J, Koidis P, Geurtsen W. Comparative characterization of STRO-1(neg)/CD146(pos) and STRO-1(pos)/CD146(pos) apical papilla stem cells enriched with flow cytometry. *Arch Oral Biol* 2013; **58**: 1556-1568 [PMID: 23871383 DOI: 10.1016/j.archoralbio.2013.06.018]
152 **Yamaza T**, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, Wang S, Shi S. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. *Stem Cell Res Ther* 2010; **1**: 5 [PMID: 20504286 DOI: 10.1186/scrt5]
153 **Kerkis I**, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, Caplan AI, Cerruti HF. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. *Cells Tissues Organs* 2006; **184**: 105-116 [PMID: 17409736 DOI: 10.1159/000099617]
154 **Miura M**, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 2003; **100**: 5807-5812 [PMID: 12716973 DOI: 10.1073/pnas.0937635100]
155 **Yamaza T**, Alatas FS, Yuniartha R, Yamaza H, Fujiyoshi JK, Yanagi Y, Yoshimaru K, Hayashida M, Matsuura T, Aijima R, Ihara K, Ohga S, Shi S, Nonaka K, Taguchi T. *In vivo* hepatogenic capacity and therapeutic potential of stem cells from human exfoliated deciduous teeth in liver fibrosis in mice. *Stem Cell Res Ther* 2015; **6**: 171 [PMID: 26358689 DOI: 10.1186/s13287-015-0154-6]
156 **Bi R**, Lyu P, Song Y, Li P, Song D, Cui C, Fan Y. Function of Dental Follicle Progenitor/Stem Cells and Their Potential in Regenerative Medicine: From Mechanisms to Applications. *Biomolecules* 2021; **11** [PMID: 34356621 DOI: 10.3390/biom11070997]
157 **Zhang Q**, Nguyen AL, Shi S, Hill C, Wilder-Smith P, Krasieva TB, Le AD. Three-dimensional spheroid culture of human gingiva-derived mesenchymal stem cells enhances mitigation of chemotherapy-induced oral mucositis. *Stem Cells Dev* 2012; **21**: 937-947 [PMID: 21689066 DOI: 10.1089/scd.2011.0252]
158 **Ansari S**, Chen C, Xu X, Annabi N, Zadeh HH, Wu BM, Khademhosseini A, Shi S, Moshaverinia A. Muscle Tissue Engineering Using Gingival Mesenchymal Stem Cells Encapsulated in Alginate Hydrogels Containing Multiple Growth Factors. *Ann Biomed Eng* 2016; **44**: 1908-1920 [PMID: 27009085 DOI: 10.1007/s10439-016-1594-6]

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REVIEW

Immunomodulation: The next target of mesenchymal stem cell-derived exosomes in the context of ischemic stroke

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Abstract

Ischemic stroke (IS) is the most prevalent form of brain disease, characterized by high morbidity, disability, and mortality. However, there is still a lack of ideal prevention and treatment measures in clinical practice. Notably, the transplantation therapy of mesenchymal stem cells (MSCs) has been a hot research topic in stroke. Nevertheless, there are risks associated with this cell therapy, including tumor formation, coagulation dysfunction, and vascular occlusion. Also, a growing number of studies suggest that the therapeutic effect after transplantation of MSCs is mainly attributed to MSC-derived exosomes (MSC-Exos). And this cell-free mediated therapy appears to circumvent many risks and difficulties when compared to cell therapy, and it may be the most promising new strategy for treating stroke as stem cell replacement therapy. Studies suggest that suppressing inflammation *via* modulation of the immune response is an additional treatment option for IS. Intriguingly, MSC-Exos mediates the inflammatory immune response following IS by modulating the central nervous system, the peripheral immune system, and immunomodulatory molecules, thereby promoting neurofunctional recovery after stroke. Thus, this paper reviews the role, potential mechanisms, and therapeutic potential of MSC-Exos in post-IS inflammation in order to identify new research targets.

Key Words: Mesenchymal stem cells; Exosomes; Ischemic stroke; Immunomodulation; Inflammation; Exosome therapy

Core Tip: Mesenchymal stem cell-derived exosomes (MSC-Exos) are an emerging strategy for treating ischemic stroke (IS) and have demonstrated certain achievements in animal studies. Here, we review and discuss the mechanisms of MSC-Exos in treating IS through immunomodulation, the current responses to the clinical limitations of MSC-Exos therapy, and the issues that need to be addressed in future MSC-Exos research.

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INTRODUCTION

Stroke is one of the leading causes of death and permanent disability on a global scale; ischemic stroke (IS) accounts for approximately 80% of stroke cases[1]. Currently, the mainstay of acute treatment for IS is limited to reperfusion by intravenous recombinant tissue fibrinolytic activator (tPA, thrombolysis) and rapid recanalization utilizing devices (thrombectomy)[2]. In clinical practice, however, the thrombolytic treatment conditions are strictly limited to presentation within 4.5 h of symptom onset[3]. Although the therapeutic window for thrombectomy has been extended to 24 h, there may be a risk of cerebral hemorrhage, occlusion after revascularization, and over-perfusion brain injury[4,5]. In recent years, other treatments that researchers have actively explored have also been prevented from being implemented on a large scale in clinical practice due to a variety of disadvantages. For instance, hypothermia treatment may reduce body metabolism while affecting neuronal death mechanisms, resulting in increased immunosuppression and susceptibility to infectious complications[6]. Prophylactic antibiotic treatment can decrease the incidence of infectious complications. However, antibiotic therapy is targeted, and broad-spectrum antibiotics can affect the body's normal flora if they are misused, which may increase organismal resistance[7]. By 2050, there will be more than 200 million stroke survivors and almost 300 million disability-adjusted life-years, 25 million new strokes, and 13 million deaths from stroke annually[1]. Therefore, there is a pressing need to discover effective treatments for IS that can be administered on a large clinical scale.

In acute stroke management, time is brain. The focus of stroke research should be on extending the time window for treatment. Examples include early measurement of immune biomarkers[8], improved efficiency of pre-hospital emergency transport[9], improved levels of care[10], and stem cell transplantation therapy[11]. Among these, stem cell transplantation therapy, which can extend the treatment window for IS to seven days, has become a hot research topic[11]. This also offers promising treatment options for patients outside the golden treatment period. MSCs are among the most hopeful candidates for stem cell therapy compared to other types due to their comprehensive source, ease of culture, pluripotent differentiation, immune tolerance, high survival rate, and strong paracrine effects [11-13]. It has previously been proved that nutrient factors and extracellular vesicles (EVs) secreted in situ by stem cells after transplantation enter the damaged brain and exert immunomodulatory, neuroprotective, angiogenic, and neural restructuring effects[13,14]. This phenomenon is known as the paracrine response (also called the "bystander" effect) and is the main mechanism by which stem cell's function. In comparison, exosomes are key effectors in the paracrine response of stem cells[14]. Mesenchymal stem cell-derived exosomes (MSC-Exos) therapy applied to stroke is superior to cell therapy in biodistribution, stability, safety, and development potential while ensuring therapeutic efficacy as an alternative therapy to stem cells.

In addition to the problem of a narrowing treatment window, the poor prognosis of IS is another pressing issue. Immunosuppression is the important cause of IS patients' poor prognosis and increased susceptibility. The inflammatory response underlies ischemic tissue damage. MSC-Exos, a highly promising treatment modality for brain injury, can effectively reduce neuroinflammatory reactions by modulating the immune system to promote recovery[15,16]. This paper reviews and discusses the immunomodulatory effects of MSC-Exos at the cellular and molecular levels following IS, as well as its application in therapy, in order to serve as a reference for future research and treatment.

IMMUNE RESPONSE AFTER IS

IS is caused by thrombosis or embolism, which could interrupt blood flow to the brain. After acute ischemic events, blood stagnation and altered hemodynamics restrict the availability of oxygen and glucose [oxygen-glucose deprivation (OGD). Then brain cell metabolism shifts from the oxidative phosphorylation to high levels of glycolysis, producing excess lactic acid[17,18]. Excessive accumulation

of lactic acid is able to trigger tissue acidosis, edema, blood-brain barrier (BBB) dysfunction, and extensive necrosis[18]. Firstly, once the Na^+/K^+ ATPase pump is affected, there will be an inward flow of Na^+ and an outward flow of K^+, which depolarizes the neuronal plasma membrane and promotes the release of excitatory neurotransmitters (including glutamate)[18-20]. Excess glutamate activates the N-methyl-D-aspartate receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor, thereby leading to cytotoxicity and cell death[19,21,22]. Next, an extracellular Ca^{2+} inward flow occurs after affecting the Ca^{2+} pump, which causes a dramatic rise in intracellular Ca^{2+}. Ca^{2+} overload activates calcium-dependent proteases, lipases, DNAases, kinase phosphatases, endonucleases, and other death signals, inducing ischemic core cell death[20,23]. Additionally, the Ca^{2+} influx activates nitric oxide synthase (iNOS), which subsequently generates oxygen radicals and peroxynitrite (ONOO-), causing oxidative stress in neural tissue[24]. Meanwhile, the depletion of ATP production and overproduction of reactive oxygen species (ROS) leads to mitochondrial dysfunction, further exacerbating oxidative stress [22,25]. In summary, OGD results in subsequent energy disturbances, lactic acidosis, cellular excitotoxicity, and oxidative stress, ultimately leading to brain cell damage or death. This is the initial step of ischemia-induced damage, which triggers the subsequent cascade responses. Injured/dying cells emit "danger signals" and thus activate the immune system (Figure 1).

Once the immune system is activated, immune cells enter the brain parenchyma sequentially. Microglia (MG), as the resident macrophages of the central nervous system (CNS), are the first to detect ischemia and rapidly activate in response[26,27]. MG recognizes "danger signals" [danger-associated molecular patterns (DAMPs)] released by dying and dead cells, primarily *via* the expressions of Toll-like receptors (TLR) and scavenger receptors. Then, the TLRs and scavenger receptors are activated, triggering a series of inflammatory events[28-30]. MG has been classified into two polarized phenotypes, including classical activation (pro-inflammatory, M1) and alternative activation (anti-inflammatory, M2). Anti-inflammatory cytokines [such as interleukin (IL)-4, IL-13, IL-10, and transforming growth factor (TGF)-β] activate the M2 phenotype. The M2 cells promote translocation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and proliferation-activated receptor gamma, and promote the secretion of anti-inflammatory IL-10, IL-4, TGF-β cytokines and growth factors (such as brain-derived neurotrophic factor and vascular endothelial growth factor) to suppress inflammation and enhance tissue repair[31,32]. In contrast, lipopolysaccharide (LPS) and interferon gamma (IFN-γ) activate the M1 phenotype. The M1 cells promote the transcriptional activation of nuclear factor-κB (NF-κB), a member of the signal transducer and activator of the transcription family (STAT), and promote the production of pro-inflammatory mediators like IL-12, tumor necrosis factor (TNF)-α, IL-6, IL-1β and NO, leading to the secondary brain damage[31,33,34]. Meanwhile, the chemokines and cytokines released by M1-MG and adhesion molecules highly expressed on endothelial cells can recruit peripheral blood leukocytes (including neutrophils, monocytes, and lymphocytes) to infiltrate the brain parenchyma, thereby mediating the adaptive immune response[28,30,31]. In the acute phase of brain injury, the M1 phenotype appears to predominate, whereas MG favors the M2 phenotype in the later stages. In addition, neurons can control MG activation by releasing "on" and "off" signals. MG is able to quickly recognize the "eat me" (CX3CL1) or "don't eat me" (*e.g.*, CD47-SIRPα and CD200-CD200R) signal on a neuron and engulf the live ischemic neurons[35]. In the same way like MG, macrophages can be polarized into two phenotypes, M1 and M2. The two are often described as MG/macrophages, because their roles in stroke are mostly similar[36]. However, in contrast, the main inflammatory factors produced by both are skewed. MGs secrete relatively high levels of ROS and TNF-α, while macrophages produce relatively high levels of IL-1β[37].

Astrocytes (Ast) are among the first brain cells to be activated after an ischemic event. Ast undergoes a dramatic transformation called "reactive astrocytosis" after ischemic injury, forming glial scarring[38]. Similar to MG, the harmful or beneficial effects of reactive Ast depend on the different phenotypes of Ast (neuronal toxicity phenotype A1 and neuroprotective phenotype A2)[38,39]. In addition to the activation of Ast by DAMPs, there is growing evidence regarding the importance of MG-Ast crosstalk for activating Ast. MG activation, followed by the release of IL-1α, TNF-α and complement component subunit 1q, induces the activation of A1-type reactive Ast[38,40]. A1-Ast secrete pro-inflammatory mediators, like IL-6, TNF-α, IL-1α, IL-1β, IFN-γ, NO, matrix metalloproteinases (MMP), superoxide and ONOO-, inducing neuron and oligodendrocyte death[38,41]. MG also induces the A2 phenotype of Ast and attenuates the inflammatory response. Li *et al*[42] have reported that Zinc finger E-box binding homeobox 1 (ZEB1) was highly expressed in MG of the ischemic hemisphere after experimentally induced strokes[42]. ZEB1 overexpression mediates the MG response primarily through a TGF-β1-dependent pathway and subsequently reduces CXCL1 production in Ast, thereby reducing neutrophil infiltration in the brain parenchyma. Likewise, Ast also can regulate the phenotype and function of MG through crosstalk between Ast and MG[43]. Thus, when the brain is disturbed, MG and Ast seem to respond as a unit.

Different from other immune cells, the number of lymphocytes infiltrating into the stroke brain is relatively small[27]. T lymphocytes can enter the brain hours after a stroke and are preferentially accumulated at the edge of the lesion[44]. The T cells infiltrating into the ischemic tissue mainly comprise $CD8^+$ cytotoxic T lymphocytes (CTLs), $CD4^+$ T helper cells (Ths), and regulatory T cells (Tregs) [45]. Infiltrating MG/macrophages may stimulate the differentiation of activated $CD4^+$ T cells into Th1

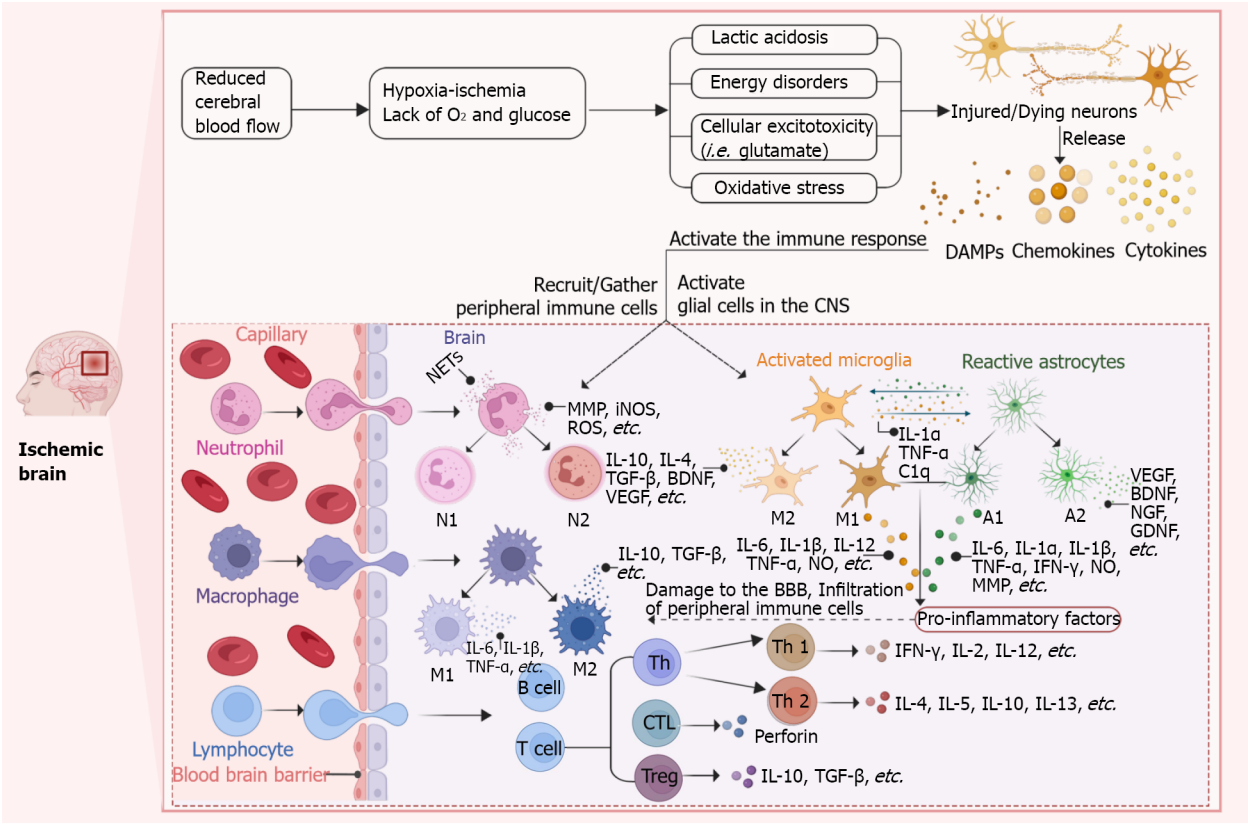

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Figure 1 Schematic representation of the immune response after ischemic stroke. After ischemic stroke (IS), the blood-brain barrier (BBB) is disrupted and the central nervous system (CNS) and the peripheral immune system are able to interact with each other. The cerebral blood flow is significantly reduced immediately after IS, which limits the availability of glucose and oxygen. The initial ischemic event leads to energy disturbances, acidosis, cellular excitotoxicity and oxidative stress ultimately resulting in neuronal damage or death and subsequent activation of the immune response. Dying/dead neurons release "danger signals" such as danger associated molecular patterns (DAMPs), cytokines and chemokines to recruit and activate peripheral immune cells (neutrophils, macrophages and lymphocytes) and activate glial cells (microglia and astrocytes) in the central CNS. Activated glial cells release a range of cytokines and chemokines that also recruit peripheral immune cells into the brain parenchyma and further destroy the BBB. Activated cells polarize into different cell phenotypes or subtypes to secrete pro-inflammatory or anti-inflammatory factors that act to damage or protect. (Figure created with BioRender.com). DAMP: Danger associated molecular patterns; CNS: Central nervous system; NET: Neutrophil extracellular trap; MMP: Matrix metalloproteinases; iNOS: Influx activates nitric oxide synthase; ROS: Reactive oxygen species; IL: Interleukin; TGF-β: Transforming growth factor-β; BDNF: Brain-derived neurotrophic factor; VEGF: Vascular endothelial growth factor; TNF-α: Tumor necrosis factor-α; NGF: Nerve growth factor; GDNF: Glial cell-derived neurotrophic factor; IFN-γ: Interferon-γ; BBB: Blood-brain barrier; CTL: Cytotoxic T lymphocyte; C1q: Component subunit 1q.

or Th2 cells[46]. Th1 cells are able to secrete pro-inflammatory factors like IFN-γ, IL-2, and IL-12 to exacerbate inflammation. In contrast, Th2 cells produce anti-inflammatory factors, such as IL-4, IL-5, IL-10, and IL-13, to suppress inflammation[47]. CTLs directly or indirectly kill neurons and aggravate brain damage through cell interactions and the release of perforin after antigen-dependent activation[48]. Tregs exert their protective effects mostly by inhibiting IL-1β and TNF-α through the expression of IL-10 [47,49]. The role of B lymphocytes in the immunology of stroke is not clear yet. Whereas, some studies have observed the local production of corresponding antibodies in the cerebrospinal fluid of stroke patients, indicating that B lymphocytes are indeed present in the ischemic brain and they may be involved in post-ischemic immunological events[50].

Neutrophils are the initial blood-derived immune cells to cross the BBB and invade ischemic tissues, and they can be detected as soon as 1 h after the event[51]. Neutrophils are activated and recruited to the injured brain parenchyma by inflammatory factors produced from some activated glial cells and dying neurons, and adhesion molecules expressed by endothelial cells (*e.g.*, intercellular adhesion molecule 1, P-selectin and E-selectin)[27,52,53]. Traditionally, the neutrophil aggregation has been considered detrimental to stroke. After infiltration into ischemic tissues, activated neutrophils produce inflammatory factors, such as MMP, iNOS, and ROS, and form neutrophil extracellular traps (NETs) to increase BBB permeability and exacerbate inflammation[54-56]. In addition, the accumulation of neutrophils can further block local blood flow, resulting in "no reflux" of the microcirculation[57]. Neutrophils also exhibit two kinds of phenotypes, comprising N1 (pro-inflammatory) and N2 (anti-inflammatory) phenotypes. Neutrophil's different phenotypes may shape other cellular effector functions and be cleared by phagocytosis of MG/macrophages[58,59].

In conclusion, the post-stroke ischemic environment induces immune cells to polarize into different phenotypes or type, acting either protectively or destructively. Hence, it is probably a promising mean to affect the immune cell heterogeneity and improve the post-stroke inflammatory environment.

EXOSOMES AS A REPLACEMENT THERAPY FOR MSCS ON IS

All above, it is clear that the immune responses following IS can influence the development of ischemic brain injury. Anti-inflammatory and immunomodulatory therapies have shown beneficial effects on several experimental stroke models[60]. Among them, MSCs transplantation is one of the most important therapeutic tools involved in regulating immunity and repairing ischemic tissues in clinical practice[61]. Initially, researchers have assumed that the primary mechanism of MSCs transplantation mainly involved in MSC's ability to differentiate into parenchymal cells to repair and replace injured tissues. However, many preclinical studies suggested that most MSCs were confined to the liver, spleen, and lungs, and only a few MSCs could reach the injury site, surviving and differentiating into neurons [62,63]. Interestingly, despite most transplanted MSCs stagnate in the organ, this does not prevent the therapeutic effect of MSCs transplantation. Thus, the distal therapeutic effect after transplantation of MSCs may be primarily attributable to the paracrine mechanism of MSCs[63]. MSC-Exos mainly mediate the paracrine secretion of MSCs. Exosomes are EVs with a single membrane structure of 30-150 nm in diameter, carrying proteins, lipids, nucleic acids (DNA, mRNA, miRNA, lncRNA, circRNA), and other substances[64]. When exosomes are circulating, the contents encapsulated within them can be delivered to target cells, mediating intercellular communication and regulating the function of the target cells[64,65]. This is essentially the role of the miRNAs contained by exosomes. MiRNAs are endogenous hairpin-loop structured non-coding RNAs, primarily binding to mRNA in specific ways to influence post-transcriptional events and regulate cellular behavior[66].

Furthermore, there are multiple advantages of transplanting exosomes rather than the entire "factory" (cell) into the body: (1) In terms of biodistribution, as nano-scale cellular secretions that could escape the phagocytosis of macrophages and readily cross the BBB to reach the brain parenchyma, they are considered to be natural therapeutic agents and innate drug delivery system for brain diseases[67]; (2) In terms of stability, exosomes have a stable bimolecular phospholipid structure that prevents the contents' biological activity from being broken down by extracellular hydrolytic enzymes[64]; (3) In terms of safety, compared to MSCs transplantation therapy, the cell-free therapy can avoid cell-mediated adverse effects, such as tumor formation, coagulation dysfunction, and infarction due to vascular occlusion[16]; and (4) In terms of development potential, exosomes can be enriched in large quantities within the culture medium (mass production) and easily retouched/retrofitted (controllable). Moreover, some studies comparing the therapeutic effects of MSC-Exos with MSCs in stroke rat models suggest that MSC-Exos treatment is indeed superior to treatment with MSCs themselves[68,69]. For above reasons, we believe that MSC-Exos is a crucial effector of MSCs to exert their immunomodulatory effects. Together with its unique advantages, MSC-Exos is expected to be a replacement therapy for MSCs in the treatment of stroke.

MSC-EXOS MODULATES IMMUNE RESPONSE AFTER IS

Recently, numerous studies have shown that MSC-Exos can promote recovery after stroke, *via* modulating the innate and adaptive immune responses activated after IS[70-72]. Firstly, MSC-Exos is able to regulate cell differentiation, activation, proliferation, and intercellular communication by delivering functional molecules to cells involved in immunity, for example, MG, Ast, macrophages, neutrophils, lymphocytes, dendritic cells (DCs), *etc.* (Table 1). There are three primary forms of action: (1) Through the signaling molecules on its surface as ligands binding to specific receptors on the target cell, the intracellular signaling pathways are regulated; (2) *via* fusing with the corresponding target cell membrane and releasing the contents into the recipient cell; and (3) by entering the target cell in the form of endocytosis and bringing the active factors into the cell[73]. Secondly, MSC-Exos can also mediate the immune response by down-regulating pro-inflammatory factors and/or up-regulating anti-inflammatory factors (Table 2).

MSC-Exos regulate the immune response through cells

MSC-Exos and CNS: MG is firstly activated after IS, as an immune sentinel of the CNS, exerting neuroprotective or neurotoxic effects[27,74]. A therapeutic strategy balancing the two polarization states of MG may become a future adjunctive stroke therapy. One study used protein blotting to analyze TLR-2, TLR-4 and TLR-6 levels in MG of ischemia/reperfusion (I/R) mice and found that the TLR/NF-κB pathway was activated in MG after an ischemic event, leading to the secretion of pro-inflammatory factors (IL-1β, TNF-α, IL-6, *etc.*) and that this signaling pathway was important in promoting M1 transformation and exacerbating the inflammatory response[75]. TLRs are pattern recognition receptors

Table 1 Mesenchymal stem cell-derived exosomes target cells to mediate immune responses on ischemic stroke

Origin	Targeted cells	Administration/cultivation routes	Pathways/factors involved	Function	Ref.
Human umbilical cord-derived mesenchymal stem cell exosome miR-26b-5p	Microglia	Tail vein injection and microglia co-culture	TLR signaling pathway	Balance microglia polarization	[75]
Bone marrow-derived mesenchymal stem cell exosome miR-182-5p	Microglia	Inject into the brain	TLR4/NF-κB		[81]
Mesenchymal stem cell exosome miR-223-3p	Microglia	Tail vein injection and BV-2 microglia co-culture	CysLT2R-mediated signaling pathway		[87,89]
Mesenchymal stem cell exosome miR-26a-5p	Microglia	Tail vein injection and BV-2 microglia co-culture	CDK6		[97]
Human umbilical cord-derived mesenchymal stem cell exosome miR-146a-5p	Microglia	Tail vein injection	IRAK1/TRAF6		[85]
Bone marrow-derived mesenchymal stem cell exosomes	Microglia	Tail vein injection	NLRP3 inflammasome		[99]
Bone marrow-derived mesenchymal stem cell exosome lncRNA H19	Microglia	BV-2 microglia co-culture	JAK/STAT		[100]
Adipose stem cell-derived exosome miR-30d-5p	Microglia	Tail vein injection and primary microglia co-culture	Autophagy		[91]
Mesenchymal stem cell exosome miR-542-3p	Neuroglia	Inject into paracele of mice	TLR signaling pathway	Mitigate OGD-induced glial cell damage	[71]
Mesenchymal stem cell exosomes	Astrocyte	Ventricular injection and astrocyte co-culture	Nrf2-NF-κB	Modulate astrocyte activation and ameliorate reactive astrogliosis	[104,105]
Bone marrow-derived mesenchymal stem cell exosome miR-138-5p	Astrocyte	Astrocyte co-culture	LCN2		[111]
Mesenchymal stem cell exosome miR-133b	Astrocyte	Tail vein injection	CTGF/RhoA		[118,119]
Human adipose-derived mesenchymal stem cell exosomes	Neutrophil	Neutrophil co-culture	IL-6	Increase neutrophil lifespan and enhance neutrophil phagocytosis	[122]
Wharton's jelly-derived mesenchymal stem cell exosomes	Neutrophil	Neutrophil co-culture	_		[123]
Adipose-derived mesenchymal stem cell-derived exosomes	Macrophage	Macrophage co-culture	MafB and Stat6	Balance macrophage polarization	[129]
Adipose-derived mesenchymal stem cell-derived exosomes	Macrophage	THP-1 cell co-culture	ROCK1/PTEN		[130]
Human adipose-derived mesenchymal stem cell exosomes	T-lymphocyte	T-lymphocyte co-culture	Markers	Inhibition of lymphocyte activation and proliferation	[135]
Bone marrow-derived mesenchymal stem cell-derived exosomes	B-lymphocyte	T-lymphocyte/B-lymphocyte co-culture	Specific mRNAs		[70]
Adipose-derived mesenchymal stem cell-derived exosomes	Dendritic cell	Dendritic cell co-culture	_		[142]

TLR: Toll-like receptors; NF-κB: Nuclear factor-κB; CysLT2R: Cysteinyl leukotriene receptor 2; LCN2: Lipid chainlipoprotein-2; JAK: Janus kinase; STAT: Signal transducer and activator of transcription; IRAK1: Interleukin-1 receptor-associated kinase 1; TRAF6: TNF receptor-associated factor 6; Nrf2: Nuclear factor erythroid 2-related factor 2; RhoA: Ras homolog gene family member A; CTGF: connective tissue growth factor; IL-6: Interleukin-6; ROCK1: Rho

associated coiled-coil containing protein kinase 1; PTEN: Phosphatase and tensin homolog; _: Refers to studies without detail the pathways

Table 2 Important immunological factors and their impact

Inflammatory mediators	Impacts	End of MSC-Exos transplantation/culture	Ref.
TNF-α	Pro-inflammatory	Decline	[71,75,81,85,91,100,104,111,130,140,141,143]
IL-1β	Pro-inflammatory	Decline	[81,85,100,104,111,140,141]
IL-6	Pro-inflammatory	Decline	[71,75,81,85,91,100,111,122,130,140-143]
iNOS	Pro-inflammatory	Decline	[81,91]
IFN-γ	Pro-inflammatory	Decline	[135]
IL-8	Pro-inflammatory	Decline	[130]
NLRP3	Pro-inflammatory	Decline	[99,145]
CysLT2R	Pro-inflammatory	Decline	[87,89]
CCL-2	Pro-inflammatory	Decline	[75]
MCP-1	Pro-inflammatory	Decline	[71,81]
IL-4	Anti-inflammatory	Raise	[91]
IL-10	Anti-inflammatory	Raise	[91,100,130,142,143]
TGF-β	Anti-inflammatory	Raise	[130,142]

TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8; iNOS: Influx activates nitric oxide synthase; IFN-γ: Interferon gamma; CysLT2R: Cysteinyl leukotriene receptor 2; CCL-2: C-C motif ligand 2; MCP-1: Monocyte chemotactic protein; IL-4: Interleukin-4; IL-10: Interleukin-10; TGF-β: Transforming growth factor-β; NLRP3: NLRP3 inflammasome; MSC-Exos: Mesenchymal stem cell-derived exosomes.

widely expressed on the surface of immune cells and play a key role in the immune response. NF-κB is a key regulator of the immune response and is intricately involved in MG/macrophage M1 and M2 phenotypic signaling[31,76,77]. Various miRNAs encapsulated in exosomes can regulate the expression of TLRs on MG surface, which act on NF-κB to influence MG polarization[29,30,78]. It has been reported that Cholesterol 25-hydroxylase (CH25H) is significantly increased during inflammation and contributes to the immune response by recruiting Iba-1-positive MG and activating TLR-3[79]. Meanwhile, an experiment used microarray to analyze the expression differences of miRNAs in ischemic brain tissue after exosome treatment, and found that miR-26b-5p expression increased significantly after exosome treatment and could target CH25H in MG to inactivate the TLR pathway to inhibit M1 polarization[75]. Besides, miR-542-3p prevents the expression of pro-inflammatory factors and the production of ROS by post-ischemic activated glial cells through inhibiting TLR[71]. miR-202-3p [80], miR-182-5p[81], MiR-181c[82], and miR-1906[83] also play a role in inhibiting M1 polarization through downregulation of TLR expression. Also, in a further explanation of the potential mechanism of miRNA-mediated TLR/NF-κB pathway, Liu *et al*[84] have documented that miR-216a-5p activates the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling cascade through inhibition of TLR4/NF-κB, enabling the M1 to M2 phenotypic shuttle[84]. Some data also suggest that miR-145-5p downregulates inflammatory responses by inhibiting the IL-1 receptor-associated kinase 1 (IRAK1)/TNF receptor-associated factor 6 (TRAF6) signaling pathway to reduce and increase the amount of M1-MG and M2-MG, respectively[85]. In contrast, overexpression of IRAK1 and TRAF6 is involved in the activation of TLR/NF-κB pathway and promotes the release of proinflammatory factors[78,85,86]. Hence, miR-145-5p may indirectly affect the TLR/NF-κB pathway by inhibiting the IRAK1/TRAF6 pathway. Exploring the crosstalk between TLR/NF-κB and other pathways may better interfere with MG polarization. As described above, exosome miRNAs acting on the TLR/NF-κB pathway and its upstream/downstream signaling pathways could influence MG phenotype as well as the expression of pro/anti-inflammatory factors to improve inflammation.

In the regulation of MG polarization, the TLR/NF-κB pathway has been studied the most. However, in stroke, MG polarization is complex and actually regulated by multiple factors. Thus, other pathways affecting the activation state of MG are discussed below. miR-223 is one of the most abundant miRNAs in MSCs and their exosomes. In vivo and *in vitro* experiments have revealed that miR-223-3p down-regulates the transcription and expression of Cysteinyl leukotriene receptor 2 (CysLT2R) to induce a conversion from deleterious M1 to beneficial M2 phenotype[87]. CysLTs secreted by dying/dead cells

are potent medium for inflammation. They are activated in various cell types during brain injury, further exacerbating the development of inflammation[88]. Zhao *et al*[89] conducted an in-depth study involving the miR-223-3p inhibiting CysLT2R expression *in vivo* and in vitro. They found that miR-223-3p reversed M1 polarization by apparently downregulating the expression of ERK1/2 downstream of CysLT2R, which led to a decrease in the secretion of pro-inflammatory factors and an increase in the secretion of anti-inflammatory and neurotrophic factors, thereby slowing down inflammatory damage [89]. In addition, miR-223-3p also effectively inhibits N-methyl-leukotriene C4/Leukotriene D4 to promote M1 to switch to M2[87,90]. Through targeting the autophagy-associated proteins Beclin-1 and Atg5, miR-30d-5p greatly inhibited autophagy-mediated polarization of MG towards M1 and reduced OGD-induced inflammatory responses[91]. Notably, autophagy may exert both beneficial and detrimental effects under IS conditions, depending on the degree of autophagy[92,93]. A moderate increase in MG autophagic activity can reduce MG activation and promote MG polarization towards the M2 phenotype, exerting a neuroprotective effect. Instead, excessive autophagy exacerbates cerebral ischemic injury. It has been demonstrated that regulation of autophagic flux and exosome biogenesis in MG plays a vital role in neuronal survival under conditions of cerebral ischemia[94]. Interestingly, the similar property was also reported in Ast[95,96]. As such, balancing the autophagic flux of immune cells after stroke may be a promising target for treating stroke. Cheng *et al*[97] have demonstrated that miR-26a-5p was downregulated and CDK6 was upregulated in MSCs-derived exosomes of middle cerebral artery occlusion (MCAO) and OGD model[97]. They then hypothesized that CDK6 might be a direct target of miR-26a-5p and further confirmed the correlation between exosome miR-26a-5p and CDK6 using a luciferase reporter gene assay. The data showed that miR-26a-5p inhibited MG apoptosis and attenuated I/R injury in mice by mediating CDK6 downregulation[97]. Besides, miR-424 can also reduce ischemic brain injury by targeting key activators of the G1/S transition in MG (including CDK6, CDC25A, and CCND1) to inhibit BV-2 MG activation[98]. CDK6 seems to be a good target of miRNAs in neuroprotection. As well as alleviating inflammation, MSC-Exos can also alleviate neuronal death by regulating MG polarization to downregulate inflammatory mediators relating to pyroptosis[99]. *In vitro* data suggest that the non-coding RNA H19 carried by MSC-Exos could attenuate M1 polarization and inflammatory responses by sponging miR-29b-3p and further inhibit neuronal apoptosis[100]. miR-29b-3p may prevent ischemic-hypoxic brain injury by activating the PI3K/Akt pathway *via* downregulating the protein phosphatase and tensin homolog (PTEN)[101]. Most studies have reported routes associated with miRNAs affecting M1 polarization, while studies acting on pathways associated with M2 polarization are still lacking and deserve further exploration.

Ast, the most abundant brain cells in the CNS, plays an essential role in neuroinflammation and neuroregeneration[40,41]. Following ischemic injury, Ast is activated by DAMPs and/or MGs and undergoes a transformation known as "reactive astrogliosis"[38]. Features include hypertrophy of the shape and overexpression of glial fibrillary acidic protein (GFAP)[102]. The activated Ast phenotype matches MG and is divided into pro-inflammatory A1 and anti-inflammatory A2. Notably, recent studies have shown that the inflammatory response mediated by Ast appears to last longer and induces more damage than MG[103]. This possibility further underlines the importance of targeted inhibition of Ast activation or induction of Ast phenotypic transformation in the treatment of IS. It has been demonstrated that Nrf2-related pathways are involved in the inflammatory response of Ast[104-106]. In one study, immunofluorescence experiments were performed after *in vivo* and *in vitro* administration of MSC-Exo, respectively, and protein blots showed that MSC-Exo reduced the expression of GFAP (Ast marker), C3 (A1 marker) and ki67 (cell proliferation marker) in LPS-stimulated cultured primary hippocampal Ast[104]. Meanwhile, the data show that MSC-Exo could reverse hippocampal Ast oxidation (*e.g.*, upregulation and nuclear translocation of Nrf2) and inflammation phenotypes (*e.g.*, NF-κB activation and translocation)[104]. These results suggest that MSC-Exo can inhibit inflammation-induced Ast activation by modulating the Nrf2-NF-κB signaling pathway. Nrf2 is a regulator of redox homeostasis and a target for the induction of inflammatory responses. In brain diseases with simultaneous inflammation and oxidative stress (*e.g.*, IS), the interaction between Nrf2 and NF-κB signaling pathway is the fundamental mechanism regulating these responses[107]. miR-146a-5p, one of the most abundant cargo miRNAs in human umbilical cord-derived MSC-Exos, dramatically decreased the expression of A1 markers [C3 and lipid chain lipoprotein-2 (LCN2)) by inhibiting the NF-κB signaling cascade, thereby reversing the neurotoxic phenotype of Ast[108]. Among them, LCN2 has been identified as a potent mediator of astrocyte neurotoxicity[109]. LCN2 secreted by reactive Ast can accelerate or propagate neuronal cell death and promote the activation of resting Ast and MG[109]. Moreover, a recent study identified high LCN2 expression in a mouse transient MCAO model and detected that IS patients with higher plasma LCN2 levels were more likely to develop a post-stroke infection[110]. Overexpression of miR-138-5p negatively regulates the LCN2 expression in Ast, thereby inhibiting inflammation and reducing ischemic nerve injury[111].

More importantly, the increase of reactive Ast results in further glial scarring. In the acute phase of IS, these physical barriers can limit the inflammation spread and infarct area to maintain CNS homeostasis. However, in the recovery phase of IS, their presence may impede the circulation and neurological tissue regeneration, affecting functional recovery in late stroke[41]. It has been demonstrated that in several previous cerebral ischemia and hypoxia models, transplantation of MSCs markedly reduced reactive Ast and further eliminated glial scarring around the lesion, promoting neuronal regeneration and

relieving inflammation[112,113]. Recently, the *in vitro* studies reported that MSCs improved brain function after transplantation mainly by reducing the number of hypertrophic Ast and GFAP overexpression through inhibition of p38 MAPK, JNK, and its downstream targets p53 and STAT1 activation by paracrine factors[114]. In addition, miR-124 attenuated Ast proliferation and migration by blocking the STAT3 pathway, thereby reducing the width of glial scarring and improving neurological function [115]. Meanwhile, miR-124 may also involve in the reprogramming neuronal progenitor cells by Ast through decreasing Notch1 expression and increasing Sox2 expression[115]. As Ast and neurons originate from the same precursor cells, Ast can be reprogrammed into neurons under some specific conditions, which can be achieved, for instance, by adjusting the expression of certain specific transcription factors (including Notch1, NeuroD1, Mash1, Ascl1, *etc.*) *in vivo*[115-117]. If Ast transdifferentiated neurons homed to the ischemic lesion and replaced lost neurons, this would help limit glial scar formation and neural connectivity after injury, contributing to neural remodeling. This may be used as an alternative therapy for neuronal replenishment after stroke in the future. Furthermore, exosomes can also mediate communication between MSCs and Ast to enhance neurological recovery after stroke. MSCs communicate with Ast and neurons by releasing miR-133b-containing exosomes and transferring miR-133b into neurons and Ast to promote neuroprotection regeneration[118]. Xin *et al*[119] further showed that miR-133b shared into Ast downregulated the expression of connective tissue growth factor, thereby reducing glial scar thickness in cerebral infarction[119]. All in all, Ast may be a potential target for the intervention in stroke. However, whether the responsiveness and function of Ast should be further reduced or enhanced may depend on the duration of the ischemic lesion, the location of the Ast, and the specific subtype of Ast.

MSC-Exos and peripheral immune system

Neutrophil: Neutrophils are the first peripheral immune cells to infiltrate into the brain parenchyma crossing the damaged BBB. Neutrophil infiltration after IS is now believed to be detrimental to stroke [27,54,56]. MSC-Exos can mitigate the harmful effects of neutrophils in several ways. Firstly, MSC-Exos can reduce neutrophil infiltration and inhibit neutrophil respiratory burst, thereby decreasing the expression of inflammatory mediators, including IL-1b, IL-6, and TNF-α, as well as suppressing the production of ROS in neutrophils[120]. Also, further studies have revealed that MSC-Exos may prevent the subsequent recruitment of monocytes/macrophages and lymphocytes after reducing neutrophil infiltration at the cerebral ischemia site[121]. Secondly, MSC-Exos inhibits neutrophil apoptosis and enhances neutrophil phagocytosis, then contributing to the clearance of cellular debris and eliminating inflammation and infection. This may result from the presence of IL-6 in MSC-Exos and its transfer into the neutrophil cytoplasm, which subsequently exerts an autocrine effect on neutrophils, thereby prolonging the survival of these cells and maintaining their effective function and viability to further improve the inflammatory response[122,123]. Thirdly, MSC-Exos inhibits the formation of terminal complement complexes on neutrophils *via* CD59, thus attenuating neutrophil activation and inhibiting the release of NETs and IL-17 from neutrophils[124]. Besides, human umbilical cord blood-derived MSC-derived EVs (exosomes) can also repair and enhance neutrophil mitochondrial function by transferring functional mitochondria, then reducing the formation of NETs[125]. Of greater importance, Soni *et al*[72] further investigated the differences in the regulation of neutrophil function by exosomes from different sources of MSCs. The results suggested that bone marrow-derived MSCs-derived exosomes (B-Exos) were more effective in prolonging the neutrophil lifespan. In contrast, adipose-derived MSCs (ADMSCs)-derived exosomes (A-Exos) were more prominent in increasing the phagocytic capacity of neutrophils and inhibiting the formation of NETs[72].

Macrophage: Activated macrophages are morphologically like MGs, which can be divided into neurotoxic M1 and neuroprotective M2 types[36]. Numerous experiments have demonstrated that MSC-Exos can effectively inhibit the effector function of M1 pro-inflammatory macrophages and/or promote the effector function of M2 anti-inflammatory macrophages, which contributes to alleviating the immune inflammatory response. It has been suggested that IFN regulatory factor (IRF) 5 could be reversibly induced by inflammatory stimuli in macrophages and IRF5 is associated with the plasticity of macrophage polarization (up- or down-regulation of M1 or M2 macrophage phenotypic marker expression)[126]. Overexpression of miR-22-3p promotes the polarization to macrophage M2, suppresses the inflammation, and attenuates I/R injury through downregulating IRF5, which is supported by increased expression of the M2 macrophage marker mannose receptor (CD206) and decreased expression of the M1 macrophage marker CD86[127]. B-Exos-derived miR-125a also exerts neuroprotective effects by targeting negative regulation of IRF5 to promote M2 phenotypic polarization [128]. Furthermore, A-Exos can promote M2 polarization by activating the M2 macrophage-specific transcription factors MafB and Stat6 to induce the expression of genes related to anti-inflammatory functions, supported by a mechanism that upregulates the expression of the M2 macrophage markers CD163, arginase-1 (Arg1) and CD206[129]. A-Exos also increased CD163, Arg1, CD206, TGF-β1, and IL-10 expression levels and decreased TNF-α, IL-6, and IL-8 expression levels by targeting the Rho associated coiled-coil containing protein kinase 1/PTEN pathway. The above results suggest that A-Exos may improve the inflammatory environment by promoting M2 polarization to increase the secretion of anti-inflammatory molecules and/or inhibiting M1 polarization from decreasing the

secretion of pro-inflammatory factors[130]. And similar to MG, miR-21, miR-146a, and miR-301a can also regulate macrophage polarization by inhibiting the TLR/NF-κB pathway[131-133].

Lymphocyte: First of all, T lymphocytes are at the heart of the adaptive immune system. Despite some subtypes of T lymphocytes exert a neuroprotective role in the early post-stroke phase, such as Tregs and Th2, on the whole, they have a negative impact on IS, as do neutrophils[45,134]. Accordingly, it appears to be a viable clinical treatment for stroke to modulate the differentiation, activation and function of various T lymphocyte subsets. In vitro studies indicated that A-Exos significantly inhibited the activation and proliferation of $CD4^+$ and $CD8^+$ T cells and reduced IFN-γ release, with directly immunosuppressive properties[135]. *In vivo* experiments showed that a dramatic reduction in the number of CTL was observed in a rat model of cerebral infarction injected intra-arterially with MSC-EV [136]. Another study has documented that MSC-Exos also promoted the Treg proliferation and induced the conversion of Th1 to Th2 by enhancing intracellular IL-10 and TGF-β secretion, thereby boosting its immunosuppressive capacity[137]. Soni *et al*[72] have further explored and found that MSC-Exos containing miR-146, miR-155, miR-21, and miR-29 may regulate the activation pathways of Th1 and Th2 [72]. And they showed that different sources of MSC-Exos all inhibited the proliferation of T lymphocytes. But in comparison, Wharton Jelly-derived exosomes and B-Exos had a better inhibitory capacity than A-Exos[72]. Additionally, DCs activate T cells through delivering co-stimulatory molecules, such as CD80 and CD86, to naive T cells[138]. However, MSC-Exos can reduce T-lymphocyte activity, increase IL-10 and TGF-β secretion, and reduce IL-6 secretion by inhibiting the maturation and differentiation of DCs[135]. Next, activation and isotype-transformed B-lymphocyte infiltration contribute to poor outcomes after IS. It has been previously reported that MSCs can reverse the unfavorable outcome by inhibiting B lymphocyte activation, proliferation, differentiation, and chemotactic response[139]. Recent studies have demonstrated that when co-cultured with lymphocytes derived from healthy human peripheral blood, B-Exos significantly inhibited lymphocyte proliferation and immunoglobulin M production, particularly exhibiting effects on B lymphocyte-specific mRNA expression[70].

MSC-Exos regulate the immune response through inflammatory mediators

In addition to immune cells, changes in inflammatory mediators, such as cytokines and chemokines, are also observed in ischemic area. Among them, TNF-α, IL-1β, and IL-6 are the more typical pro-inflammatory factors, and their expression is significantly upregulated after ischemic events. MSC-Exos containing lncRNA ZFAS1[140], lncRNAH19[100], miR21-3p[141], miR-146a-5p[85], miR-138-5p[111], and miR182-5p[81] was able to reduce immunosuppression by downregulating the secretion of TNF-α, IL-1β, and IL-6. Of these, lncRNA ZFAS1 and lncRNAH19 may be associated with the competitive binding of miR-15a-5p and miR-29b-3p[100,140]. MSC-Exos also down-regulate other pro-inflammatory factors such as IFN-γ, iNOS, and IL-8[81,91,130,135]. Apart from down-regulating pro-inflammatory factors, some MSC-Exos can up-regulate the expression of anti-inflammatory factors such as IL-10, IL-4, and TGF-β1[91,100,130,142,143]. Furthermore, some studies have shown that MSC-Exos could reduce the secretion of chemokines (*e.g.*, C-C motif ligand 2) and cellular chemotactic proteins (*e.g.*, monocyte chemotactic protein), thereby inhibiting the migration and aggregation of peripheral immune cells and alleviating the inflammatory response[71,75,81]. Inflammasomes are equally important inflammatory mediators in regulating the onset and progression of IS. NLRP3 inflammasome contains a caspase-1 precursor that cleaves to caspase-1 (Cl) upon stroke stimulation. C1 not only is a critical executioner of pyroptosis (cleave full-length GSDMD to release GSDMD N-terminus) but also can convert precursors of IL-1β and IL-18 into mature pro-inflammatory cytokines exacerbating inflammation[144]. Liu *et al*[99] found that NLRP3 inflammasome was downregulated in MCAO mice after MSC-Exos treatment, thereby reducing inflammation and pyroptosis. They also observed that MSC-Exos contributed to MG polarization towards the M2 phenotype by inhibiting NLRP3[99]. On the one hand, it may be due to the high plasticity of the MG phenotype, which can dynamically switch according to brain environmental variables[74]. On the other hand, it may be related to the amount of autophagy[91,99,145].

Collectively, MSC-Exos could improve the immune inflammatory response after IS *via* affecting the activation of MG/macrophages and Ast, reducing reactive astrocyte hyperplasia, decreasing excessive infiltration of neutrophils, balancing the functional status of T cell subsets, suppressing the proliferation of B cells, inhibiting DC maturation, and regulating the secretion of inflammatory mediators. Therefore, MSC-Exos exhibits immunomodulatory effects and may help to reduce neurological damage and promote neurological repair after IS.

FROM BENCH TO BEDSIDE: RESPONSES TO THE LIMITATIONS OF MSC-EXOS THERAPY FOR IS

To date, a growing number of studies have demonstrated the great potential of MSC-Exos in treating IS. However, lacking target ability of natural exosomes produced by MSCs has greatly limited their clinical

application[146]. Regarding exosome producing, the cell membrane invaginates to form endosomes, which in turn form multivesicular bodies (MVBs), and the MVBs finally fuse with the plasma membrane to release luminal vesicles (called exosomes) into the extracellular matrix (ECM)[147]. At present, basing on the production process of exosome, two strategies are proposed to improve the targeting ability of exosomes, comprising "cell engineering" (pre-isolation) and "exosome engineering" (post-isolation) [147] (Figure 2). For example, the cyclin (Arg-Gly-Asp-DTyr-Lys) peptide [c(RGDyK)] and the rabies virus glycoprotein (RVG) peptide have been used explicitly to target the brain. B-Exos loaded with cholesterol-modified miR-210 coupled to c(RGDyK) could bind to integrin αvβ3 on the BBB and deliver miR-210 to the site of cerebral infarction, thereby ameliorating post-stroke symptoms[148]. Additionally, c(RGDyK)-modified MSC-Exos loaded with curcumin (cRGD-Exo-cur) was used in a study, followed by tail vein injection to target the area of cerebral ischemic injury and enter neurons, MG and Ast, effectively inhibiting the inflammatory response and cellular apoptosis[146]. RVG fused with exosomes protein lysosome-associated membrane glycoprotein 2b could bind to acetylcholine receptors on the BBB and effectively deliver miR-124 to the infarct site, therefore promoting post-IS neurogenesis and reducing ischemic injury[149]. In another study, high-mobility group box 1 (HMGB1)-siRNA was loaded into RVG-modified exosomes (RVG-Exos) by electroporation and injected into an MCAO model *via* tail vein. The results showed that RVG-Exos loaded with HMGB1-siRNA was effective in reducing the level of brain apoptosis and infarct size and had the potential to target IS[150].

Next, the "low yield bottleneck" of MSC-Exos is also one of the main causes limiting its clinical application. Some researchers have illustrated that the pretreatment of MSCs appears to increase the yield of MSC-Exos. For instance, the three-dimensional porous scaffold structure increases the surface area for cell-ECM interaction, compared to the traditional two-dimensional culture of BMSCs. In addition, the three-dimensional culture more closely resembled the natural ECM conditions, providing a better environment for cell attachment and growth, thus substantially increasing the yield of MSC-Exos[151]. Some studies have indicated that cultures using microcarriers and hollow fiber bioreactor can provide cells a larger attachment area and further enhance the secretion of MSC-Exos[152,153]. Moreover, a recent study found that pretreatment of MSCs with small molecule modulators (N-methyl-dopamine and norepinephrine) tripled the production of exosomes without altering the intrinsic regenerative effects of MSC-Exos and the level of total exosomes protein expression[154].

To sum up, enhancing the targeting of exosomes by modifying them and improving the yield of exosomes by pretreating MSCs can both improve the therapeutic ability of exosomes. And further exploration of exosome improvement methods offers the possibility of transitioning from bench to bedside.

CONCLUSION

IS is a severe cerebrovascular disease that adversely affects patient's health and quality of life. A growing body of evidence suggests that the immune inflammatory response plays a critical role in pathogenesis of IS. It has emerged as a promising target for intervention in stroke therapy. After IS, the boundary between the CNS and peripheral immune system disappears due to the disruption of the BBB. Subsequently, the CNS and peripheral immune system can interact with each other, providing a unique opportunity to regulate the pathological process of IS and the repair process. At the same time, immunomodulation is one of the main mechanisms by which MSC and MSC-Exos exert their therapeutic effects on IS. MSC-Exos is expected to be an alternative therapy to MSC in treating stroke due to its parental cell-like capabilities and specific advantages. MSC-Exos exerts immunomodulatory effects mainly by affecting the inflammatory phenotype of glial cells in the CNS, inhibiting peripheral immune cell activation, proliferation, differentiation, and hyperinfilation, and regulating the secretion of immune-related molecules. Meanwhile, to complement or enhance the therapeutic suitability of exosomes, researchers are actively exploring novel methods to expand, modify or enhance their therapeutic capacity, such as modifying exosomes (to improve targeting) and pretreating MSC (to increase exosome yield).

Although the results of numerous preclinical studies have shown MSC-Exos to be one of the key breakthroughs in treating IS. However, the study of MSC-Exos in the treatment of IS is still in its infancy in clinical practice. Currently, there is only one study in the Clinical Trials Registry database to determine the effect of MSC-Exos administration on improving functional impairment after IS. This trial used the administration of miR-124-enriched isoform MSC-Exos to treat IS and entered into a phase I/II clinical trial (NCT03384433)[155]. There are many challenges to overcome to transfer MSC-Exos therapy further into the clinic: (1) Optimal duration of treatment and effective dose. Numerous studies have shown that post-IS inflammatory cells play a dual role (beneficial and detrimental) and the inhibition of the same pathway at the wrong time may exacerbate ischemic damage[27,36,38,45]. Therefore, during developing new therapeutic strategies for IS, we need to pay more attention to the duration of treatment. Interestingly, there are also cases where the timing of transplantation based on previous cellular therapies may affect the therapeutic outcome[156]. Thus, we need to further investigate the optimal timing of treatment with exosomes that may be influenced by parental cells. Most preclinical

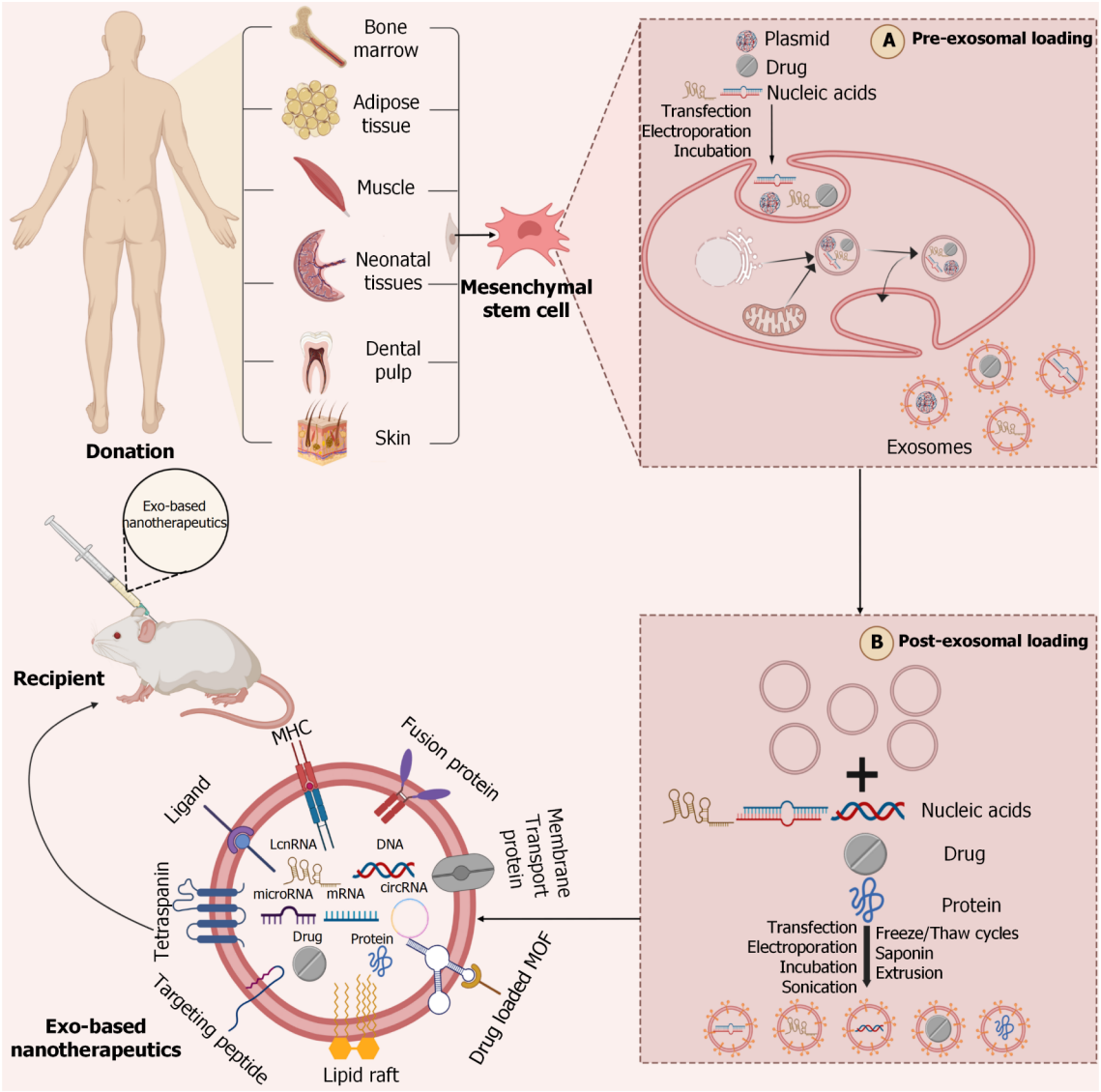

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Figure 2 Strategies to enhance the targeting of mesenchymal stem cell-derived exosomes. Multiple strategies to be done before exosomes can be successfully translated into new technologies to improve the targeting ability of donor cells and therapeutic efficacy of chemical and biomolecular drugs. Current preclinical studies are focused on parental cells' modification (pre-isolation) (A); manipulation of exosome (post-isolation) (B). (Figure created with BioRender.com).

trials have currently chosen to administer a single dose of MSC-Exos in the acute phase of the stroke while showing beneficial effects. As such, the next step should investigate the effects of delayed-time dosing compared to acute phase dosing, in order to determine the optimal timing of treatment. However, determining the optimal timing of dosing may be difficult in practice, so we could further consider multiple repeat dosing; (2) A single research direction. Current experimental studies on the immunomodulatory aspects of MSC-Exos treatment with IS have focused on MG/macrophages, while other immune-related cells or factors remain poorly studied. Furthermore, the immune response following IS results from crosstalk within and between different cell types, which is complex and chronological. However, most experimental studies have usually explored a single mechanism of action mainly in a particular cell. There is no consensus on the exact molecular mechanism of MSC-Exos treatment of IS and further in-depth studies in multiple directions are urgently needed; (3) Lack of clinical trials; and (4) Stroke models combined with relevant clinical conditions. Current studies targeting MSC-Exos for treating IS have almost always been conducted in healthy animals. Therefore, when using stroke models, it should be as close as possible to achieve the clinical situation, like hypertension, diabetes, heart disease, atherosclerosis, secondary infections, *etc.*, as these diseases may affect the formation, treatment, and prognosis of stroke. To summarize, there are still many animal experiments and clinical trials to be finished before the fact that MSC-Exos can be applied as a routine treatment for stroke. However, based on the available evidence, we believe that MSC-Exos therapy is an emerging therapeutic strategy based on cellular therapy with great potential for future use in IS treatment, particularly in immunomodulation.

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REFERENCES

1 **GBD 2019 Stroke Collaborators**. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* 2021; **20**: 795-820 [PMID: 34487721 DOI: 10.1016/S1474-4422(21)00252-0]
2 **Zerna C**, Thomalla G, Campbell BCV, Rha JH, Hill MD. Current practice and future directions in the diagnosis and acute treatment of ischaemic stroke. *Lancet* 2018; **392**: 1247-1256 [PMID: 30319112 DOI: 10.1016/S0140-6736(18)31874-9]
3 **Simon E**, Forghani M, Abramyuk A, Winzer S, Wojciechowski C, Pallesen LP, Siepmann T, Reichmann H, Puetz V, Barlinn K, Barlinn J. Intravenous Thrombolysis by Telestroke in the 3- to 4.5-h Time Window. *Front Neurol* 2021; **12**: 756062 [PMID: 34899575 DOI: 10.3389/fneur.2021.756062]
4 **Yafasova A**, Fosbøl EL, Johnsen SP, Kruuse C, Petersen JK, Alhakak A, Vinding NE, Torp-Pedersen C, Gislason GH, Køber L, Butt JH. Time to Thrombolysis and Long-Term Outcomes in Patients With Acute Ischemic Stroke: A Nationwide Study. *Stroke* 2021; **52**: 1724-1732 [PMID: 33657854 DOI: 10.1161/STROKEAHA.120.032837]
5 **Jovin TG**, Nogueira RG, Lansberg MG, Demchuk AM, Martins SO, Mocco J, Ribo M, Jadhav AP, Ortega-Gutierrez S, Hill MD, Lima FO, Haussen DC, Brown S, Goyal M, Siddiqui AH, Heit JJ, Menon BK, Kemp S, Budzik R, Urra X, Marks MP, Costalat V, Liebeskind DS, Albers GW. Thrombectomy for anterior circulation stroke beyond 6 h from time last known well (AURORA): a systematic review and individual patient data meta-analysis. *Lancet* 2022; **399**: 249-258 [PMID: 34774198 DOI: 10.1016/S0140-6736(21)01341-6]
6 **van der Worp HB**, Macleod MR, Kollmar R; European Stroke Research Network for Hypothermia (EuroHYP). Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? *J Cereb Blood Flow Metab* 2010; **30**: 1079-1093 [PMID: 20354545 DOI: 10.1038/jcbfm.2010.44]
7 **Meisel A**. Preventive antibiotic therapy in stroke: PASSed away? *Lancet* 2015; **385**: 1486-1487 [PMID: 25612859 DOI: 10.1016/S0140-6736(15)60076-9]
8 **Ng GJL**, Quek AML, Cheung C, Arumugam TV, Seet RCS. Stroke biomarkers in clinical practice: A critical appraisal. *Neurochem Int* 2017; **107**: 11-22 [PMID: 28088349 DOI: 10.1016/j.neuint.2017.01.005]
9 **Patel RAG**, White CJ. Geographic Disparities in the Treatment of Acute Stroke and the Role of Interventional Cardiologists. *JACC Cardiovasc Interv* 2020; **13**: 892-894 [PMID: 32273101 DOI: 10.1016/j.jcin.2020.02.002]
10 **Ospel JM**, Holodinsky JK, Goyal M. Management of Acute Ischemic Stroke Due to Large-Vessel Occlusion: JACC Focus Seminar. *J Am Coll Cardiol* 2020; **75**: 1832-1843 [PMID: 32299595 DOI: 10.1016/j.jacc.2019.10.034]
11 **Ziaee SM**, Tabeshmehr P, Haider KH, Farrokhi M, Shariat A, Amiri A, Hosseini SM. Optimization of time for neural stem cells transplantation for brain stroke in rats. *Stem Cell Investig* 2017; **4**: 29 [PMID: 28529944 DOI: 10.21037/sci.2017.03.10]
12 **Faghih H**, Javeri A, Taha MF. Impact of early subcultures on stemness, migration and angiogenic potential of adipose tissue-derived stem cells and their resistance to in vitro ischemic condition. *Cytotechnology* 2017; **69**: 885-900 [PMID: 28536871 DOI: 10.1007/s10616-017-0104-5]

13 **Bang OY**, Kim EH, Cha JM, Moon GJ. Adult Stem Cell Therapy for Stroke: Challenges and Progress. *J Stroke* 2016; **18**: 256-266 [PMID: 27733032 DOI: 10.5853/jos.2016.01263]

14 **Zhou Y**, Yamamoto Y, Xiao Z, Ochiya T. The Immunomodulatory Functions of Mesenchymal Stromal/Stem Cells Mediated *via* Paracrine Activity. *J Clin Med* 2019; **8** [PMID: 31336889 DOI: 10.3390/jcm8071025]

15 **Men Y**, Yelick J, Jin S, Tian Y, Chiang MSR, Higashimori H, Brown E, Jarvis R, Yang Y. Exosome reporter mice reveal the involvement of exosomes in mediating neuron to astroglia communication in the CNS. *Nat Commun* 2019; **10**: 4136 [PMID: 31515491 DOI: 10.1038/s41467-019-11534-w]

16 **Kim HY**, Kim TJ, Kang L, Kim YJ, Kang MK, Kim J, Ryu JH, Hyeon T, Yoon BW, Ko SB, Kim BS. Mesenchymal stem cell-derived magnetic extracellular nanovesicles for targeting and treatment of ischemic stroke. *Biomaterials* 2020; **243**: 119942 [PMID: 32179302 DOI: 10.1016/j.biomaterials.2020.119942]

17 **Lynch MA**. Can the emerging field of immunometabolism provide insights into neuroinflammation? *Prog Neurobiol* 2020; **184**: 101719 [PMID: 31704314 DOI: 10.1016/j.pneurobio.2019.101719]

18 **Leng T**, Shi Y, Xiong ZG, Sun D. Proton-sensitive cation channels and ion exchangers in ischemic brain injury: new therapeutic targets for stroke? *Prog Neurobiol* 2014; **115**: 189-209 [PMID: 24467911 DOI: 10.1016/j.pneurobio.2013.12.008]

19 **Wu QJ**, Tymianski M. Targeting NMDA receptors in stroke: new hope in neuroprotection. *Mol Brain* 2018; **11**: 15 [PMID: 29534733 DOI: 10.1186/s13041-018-0357-8]

20 **Xu J**, Kurup P, Zhang Y, Goebel-Goody SM, Wu PH, Hawasli AH, Baum ML, Bibb JA, Lombroso PJ. Extrasynaptic NMDA receptors couple preferentially to excitotoxicity *via* calpain-mediated cleavage of STEP. *J Neurosci* 2009; **29**: 9330-9343 [PMID: 19625523 DOI: 10.1523/JNEUROSCI.2212-09.2009]

21 **Zaghmi A**, Dopico-López A, Pérez-Mato M, Iglesias-Rey R, Hervella P, Greschner AA, Bugallo-Casal A, da Silva A, Gutiérrez-Fernández M, Castillo J, Pérez FC, Gauthier MA. Sustained blood glutamate scavenging enhances protection in ischemic stroke. *Commun Biol* 2020; **3**: 729 [PMID: 33273696 DOI: 10.1038/s42003-020-01406-1]

22 **Chamorro Á**, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol* 2016; **15**: 869-881 [PMID: 27180033 DOI: 10.1016/S1474-4422(16)00114-9]

23 **Szeto V**, Chen NH, Sun HS, Feng ZP. The role of K(ATP) channels in cerebral ischemic stroke and diabetes. *Acta Pharmacol Sin* 2018; **39**: 683-694 [PMID: 29671418 DOI: 10.1038/aps.2018.10]

24 **Love S**. Oxidative stress in brain ischemia. *Brain Pathol* 1999; **9**: 119-131 [PMID: 9989455 DOI: 10.1111/j.1750-3639.1999.tb00214.x]

25 **Casas AI**, Kleikers PW, Geuss E, Langhauser F, Adler T, Busch DH, Gailus-Durner V, de Angelis MH, Egea J, Lopez MG, Kleinschnitz C, Schmidt HH. Calcium-dependent blood-brain barrier breakdown by NOX5 limits postreperfusion benefit in stroke. *J Clin Invest* 2019; **129**: 1772-1778 [PMID: 30882367 DOI: 10.1172/JCI124283]

26 **Lambertsen KL**, Meldgaard M, Ladeby R, Finsen B. A quantitative study of microglial-macrophage synthesis of tumor necrosis factor during acute and late focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 2005; **25**: 119-135 [PMID: 15678118 DOI: 10.1038/sj.jcbfm.9600014]

27 **Gelderblom M**, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, Arumugam TV, Orthey E, Gerloff C, Tolosa E, Magnus T. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke* 2009; **40**: 1849-1857 [PMID: 19265055 DOI: 10.1161/STROKEAHA.108.534503]

28 **ElAli A**, Rivest S. Microglia Ontology and Signaling. *Front Cell Dev Biol* 2016; **4**: 72 [PMID: 27446922 DOI: 10.3389/fcell.2016.00072]

29 **Caso JR**, Pradillo JM, Hurtado O, Lorenzo P, Moro MA, Lizasoain I. Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. *Circulation* 2007; **115**: 1599-1608 [PMID: 17372179 DOI: 10.1161/CIRCULATIONAHA.106.603431]

30 **Olson JK**, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J Immunol* 2004; **173**: 3916-3924 [PMID: 15356140 DOI: 10.4049/jimmunol.173.6.3916]

31 **Jiang CT**, Wu WF, Deng YH, Ge JW. Modulators of microglia activation and polarization in ischemic stroke (Review). *Mol Med Rep* 2020; **21**: 2006-2018 [PMID: 32323760 DOI: 10.3892/mmr.2020.11003]

32 **Chen J**, Yin W, Tu Y, Wang S, Yang X, Chen Q, Zhang X, Han Y, Pi R. L-F001, a novel multifunctional ROCK inhibitor, suppresses neuroinflammation in vitro and in vivo: Involvement of NF-κB inhibition and Nrf2 pathway activation. *Eur J Pharmacol* 2017; **806**: 1-9 [PMID: 28320516 DOI: 10.1016/j.ejphar.2017.03.025]

33 **Yang S**, Wang H, Yang Y, Wang R, Wang Y, Wu C, Du G. Baicalein administered in the subacute phase ameliorates ischemia-reperfusion-induced brain injury by reducing neuroinflammation and neuronal damage. *Biomed Pharmacother* 2019; **117**: 109102 [PMID: 31228802 DOI: 10.1016/j.biopha.2019.109102]

34 **Butturini E**, Boriero D, Carcereri de Prati A, Mariotto S. STAT1 drives M1 microglia activation and neuroinflammation under hypoxia. *Arch Biochem Biophys* 2019; **669**: 22-30 [PMID: 31121156 DOI: 10.1016/j.abb.2019.05.011]

35 **Zhang Y**, Lian L, Fu R, Liu J, Shan X, Jin Y, Xu S. Microglia: The Hub of Intercellular Communication in Ischemic Stroke. *Front Cell Neurosci* 2022; **16**: 889442 [PMID: 35518646 DOI: 10.3389/fncel.2022.889442]

36 **Hu X**, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, Chen J. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 2012; **43**: 3063-3070 [PMID: 22933588 DOI: 10.1161/STROKEAHA.112.659656]

37 **Ritzel RM**, Patel AR, Grenier JM, Crapser J, Verma R, Jellison ER, McCullough LD. Functional differences between microglia and monocytes after ischemic stroke. *J Neuroinflammation* 2015; **12**: 106 [PMID: 26022493 DOI: 10.1186/s12974-015-0329-1]

38 **Liddelow SA**, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017; **541**: 481-487 [PMID: 28099414 DOI: 10.1038/nature21029]

39 **Zamanian JL**, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA. Genomic analysis of reactive astrogliosis. *J*

Neurosci 2012; **32**: 6391-6410 [PMID: 22553043 DOI: 10.1523/JNEUROSCI.6221-11.2012]
40 **Liddelow SA**, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity* 2017; **46**: 957-967 [PMID: 28636962 DOI: 10.1016/j.immuni.2017.06.006]
41 **Liu Z**, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Prog Neurobiol* 2016; **144**: 103-120 [PMID: 26455456 DOI: 10.1016/j.pneurobio.2015.09.008]
42 **Li D**, Lang W, Zhou C, Wu C, Zhang F, Liu Q, Yang S, Hao J. Upregulation of Microglial ZEB1 Ameliorates Brain Damage after Acute Ischemic Stroke. *Cell Rep* 2018; **22**: 3574-3586 [PMID: 29590624 DOI: 10.1016/j.celrep.2018.03.011]
43 **Kim S**, Son Y. Astrocytes Stimulate Microglial Proliferation and M2 Polarization In Vitro through Crosstalk between Astrocytes and Microglia. *Int J Mol Sci* 2021; **22** [PMID: 34445510 DOI: 10.3390/ijms22168800]
44 **Gill D**, Veltkamp R. Dynamics of T cell responses after stroke. *Curr Opin Pharmacol* 2016; **26**: 26-32 [PMID: 26452204 DOI: 10.1016/j.coph.2015.09.009]
45 **Hedrick SM**. T cell development: bottoms-up. *Immunity* 2002; **16**: 619-622 [PMID: 12049714 DOI: 10.1016/s1074-7613(02)00316-3]
46 **Dolati S**, Ahmadi M, Khalili M, Taheraghdam AA, Siahmansouri H, Babaloo Z, Aghebati-Maleki L, Jadidi-Niaragh F, Younesi V, Yousefi M. Peripheral Th17/Treg imbalance in elderly patients with ischemic stroke. *Neurol Sci* 2018; **39**: 647-654 [PMID: 29353353 DOI: 10.1007/s10072-018-3250-4]
47 **Filiano AJ**, Gadani SP, Kipnis J. How and why do T cells and their derived cytokines affect the injured and healthy brain? *Nat Rev Neurosci* 2017; **18**: 375-384 [PMID: 28446786 DOI: 10.1038/nrn.2017.39]
48 **Mracsko E**, Liesz A, Stojanovic A, Lou WP, Osswald M, Zhou W, Karcher S, Winkler F, Martin-Villalba A, Cerwenka A, Veltkamp R. Antigen dependently activated cluster of differentiation 8-positive T cells cause perforin-mediated neurotoxicity in experimental stroke. *J Neurosci* 2014; **34**: 16784-16795 [PMID: 25505331 DOI: 10.1523/JNEUROSCI.1867-14.2014]
49 **Liesz A**, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, Giese T, Veltkamp R. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med* 2009; **15**: 192-199 [PMID: 19169263 DOI: 10.1038/nm.1927]
50 **Prüss H**, Iggena D, Baldinger T, Prinz V, Meisel A, Endres M, Dirnagl U, Schwab JM. Evidence of intrathecal immunoglobulin synthesis in stroke: a cohort study. *Arch Neurol* 2012; **69**: 714-717 [PMID: 22371852 DOI: 10.1001/archneurol.2011.3252]
51 **Stevens SL**, Bao J, Hollis J, Lessov NS, Clark WM, Stenzel-Poore MP. The use of flow cytometry to evaluate temporal changes in inflammatory cells following focal cerebral ischemia in mice. *Brain Res* 2002; **932**: 110-119 [PMID: 11911867 DOI: 10.1016/s0006-8993(02)02292-8]
52 **Mracsko E**, Javidi E, Na SY, Kahn A, Liesz A, Veltkamp R. Leukocyte invasion of the brain after experimental intracerebral hemorrhage in mice. *Stroke* 2014; **45**: 2107-2114 [PMID: 24916913 DOI: 10.1161/STROKEAHA.114.005801]
53 **Perez-de-Puig I**, Miró-Mur F, Ferrer-Ferrer M, Gelpi E, Pedragosa J, Justicia C, Urra X, Chamorro A, Planas AM. Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol* 2015; **129**: 239-257 [PMID: 25548073 DOI: 10.1007/s00401-014-1381-0]
54 **Laridan E**, Denorme F, Desender L, François O, Andersson T, Deckmyn H, Vanhoorelbeke K, De Meyer SF. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol* 2017; **82**: 223-232 [PMID: 28696508 DOI: 10.1002/ana.24993]
55 **Allen C**, Thornton P, Denes A, McColl BW, Pierozynski A, Monestier M, Pinteaux E, Rothwell NJ, Allan SM. Neutrophil cerebrovascular transmigration triggers rapid neurotoxicity through release of proteases associated with decondensed DNA. *J Immunol* 2012; **189**: 381-392 [PMID: 22661091 DOI: 10.4049/jimmunol.1200409]
56 **Jickling GC**, Liu D, Ander BP, Stamova B, Zhan X, Sharp FR. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J Cereb Blood Flow Metab* 2015; **35**: 888-901 [PMID: 25806703 DOI: 10.1038/jcbfm.2015.45]
57 **Rolfes L**, Riek-Burchardt M, Pawlitzki M, Minnerup J, Bock S, Schmidt M, Meuth SG, Gunzer M, Neumann J. Neutrophil granulocytes promote flow stagnation due to dynamic capillary stalls following experimental stroke. *Brain Behav Immun* 2021; **93**: 322-330 [PMID: 33486002 DOI: 10.1016/j.bbi.2021.01.011]
58 **Cuartero MI**, Ballesteros I, Moraga A, Nombela F, Vivancos J, Hamilton JA, Corbí ÁL, Lizasoain I, Moro MA. N2 neutrophils, novel players in brain inflammation after stroke: modulation by the PPARγ agonist rosiglitazone. *Stroke* 2013; **44**: 3498-3508 [PMID: 24135932 DOI: 10.1161/STROKEAHA.113.002470]
59 **Xie M**, Hao Y, Feng L, Wang T, Yao M, Li H, Ma D, Feng J. Neutrophil Heterogeneity and its Roles in the Inflammatory Network after Ischemic Stroke. *Curr Neuropharmacol* 2022 [PMID: 35794770 DOI: 10.2174/1570159X20666220706115957]
60 **Levard D**, Buendia I, Lanquetin A, Glavan M, Vivien D, Rubio M. Filling the gaps on stroke research: Focus on inflammation and immunity. *Brain Behav Immun* 2021; **91**: 649-667 [PMID: 33017613 DOI: 10.1016/j.bbi.2020.09.025]
61 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736 [PMID: 19172693 DOI: 10.1038/nri2395]
62 **Gao J**, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 2001; **169**: 12-20 [PMID: 11340257 DOI: 10.1159/000047856]
63 **Oh SH**, Choi C, Chang DJ, Shin DA, Lee N, Jeon I, Sung JH, Lee H, Hong KS, Ko JJ, Song J. Early neuroprotective effect with lack of long-term cell replacement effect on experimental stroke after intra-arterial transplantation of adipose-derived mesenchymal stromal cells. *Cytotherapy* 2015; **17**: 1090-1103 [PMID: 26031742 DOI: 10.1016/j.jcyt.2015.04.007]
64 **Pegtel DM**, Gould SJ. Exosomes. *Annu Rev Biochem* 2019; **88**: 487-514 [PMID: 31220978 DOI: 10.1146/annurev-biochem-013118-111902]
65 **Zhang J**, Li S, Li L, Li M, Guo C, Yao J, Mi S. Exosome and exosomal microRNA: trafficking, sorting, and function.

Genomics Proteomics Bioinformatics 2015; **13**: 17-24 [PMID: 25724326 DOI: 10.1016/j.gpb.2015.02.001]

66 **Slota JA**, Booth SA. MicroRNAs in Neuroinflammation: Implications in Disease Pathogenesis, Biomarker Discovery and Therapeutic Applications. *Noncoding RNA* 2019; **5** [PMID: 31022830 DOI: 10.3390/ncrna5020035]

67 **Otero-Ortega L**, Laso-García F, Frutos MCG, Diekhorst L, Martínez-Arroyo A, Alonso-López E, García-Bermejo ML, Rodríguez-Serrano M, Arrúe-Gonzalo M, Díez-Tejedor E, Fuentes B, Gutiérrez-Fernández M. Low dose of extracellular vesicles identified that promote recovery after ischemic stroke. *Stem Cell Res Ther* 2020; **11**: 70 [PMID: 32075692 DOI: 10.1186/s13287-020-01601-1]

68 **Doeppner TR**, Herz J, Görgens A, Schlechter J, Ludwig AK, Radtke S, de Miroschedji K, Horn PA, Giebel B, Hermann DM. Extracellular Vesicles Improve Post-Stroke Neuroregeneration and Prevent Postischemic Immunosuppression. *Stem Cells Transl Med* 2015; **4**: 1131-1143 [PMID: 26339036 DOI: 10.5966/sctm.2015-0078]

69 **Moon GJ**, Sung JH, Kim DH, Kim EH, Cho YH, Son JP, Cha JM, Bang OY. Application of Mesenchymal Stem Cell-Derived Extracellular Vesicles for Stroke: Biodistribution and MicroRNA Study. *Transl Stroke Res* 2019; **10**: 509-521 [PMID: 30341718 DOI: 10.1007/s12975-018-0668-1]

70 **Khare D**, Or R, Resnick I, Barkatz C, Almogi-Hazan O, Avni B. Mesenchymal Stromal Cell-Derived Exosomes Affect mRNA Expression and Function of B-Lymphocytes. *Front Immunol* 2018; **9**: 3053 [PMID: 30622539 DOI: 10.3389/fimmu.2018.03053]

71 **Cai G**, Cai G, Zhou H, Zhuang Z, Liu K, Pei S, Wang Y, Wang H, Wang X, Xu S, Cui C, Sun M, Guo S, Jia K, Zhang D. Mesenchymal stem cell-derived exosome miR-542-3p suppresses inflammation and prevents cerebral infarction. *Stem Cell Res Ther* 2021; **12**: 2 [PMID: 33407827 DOI: 10.1186/s13287-020-02030-w]

72 **Soni N**, Gupta S, Rawat S, Krishnakumar V, Mohanty S, Banerjee A. MicroRNA-Enriched Exosomes from Different Sources of Mesenchymal Stem Cells Can Differentially Modulate Functions of Immune Cells and Neurogenesis. *Biomedicines* 2021; **10** [PMID: 35052749 DOI: 10.3390/biomedicines10010069]

73 **He C**, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. *Theranostics* 2018; **8**: 237-255 [PMID: 29290805 DOI: 10.7150/thno.21945]

74 **Wang G**, Zhang J, Hu X, Zhang L, Mao L, Jiang X, Liou AK, Leak RK, Gao Y, Chen J. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J Cereb Blood Flow Metab* 2013; **33**: 1864-1874 [PMID: 23942366 DOI: 10.1038/jcbfm.2013.146]

75 **Li G**, Xiao L, Qin H, Zhuang Q, Zhang W, Liu L, Di C, Zhang Y. Exosomes-carried microRNA-26b-5p regulates microglia M1 polarization after cerebral ischemia/reperfusion. *Cell Cycle* 2020; **19**: 1022-1035 [PMID: 32208888 DOI: 10.1080/15384101.2020.1743912]

76 **Oeckinghaus A**, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat Immunol* 2011; **12**: 695-708 [PMID: 21772278 DOI: 10.1038/ni.2065]

77 **Taetzsch T**, Levesque S, McGraw C, Brookins S, Luqa R, Bonini MG, Mason RP, Oh U, Block ML. Redox regulation of NF-κB p50 and M1 polarization in microglia. *Glia* 2015; **63**: 423-440 [PMID: 25331559 DOI: 10.1002/glia.22762]

78 **Wang Y**, Zhang S, Li H, Wang H, Zhang T, Hutchinson MR, Yin H, Wang X. Small-Molecule Modulators of Toll-like Receptors. *Acc Chem Res* 2020; **53**: 1046-1055 [PMID: 32233400 DOI: 10.1021/acs.accounts.9b00631]

79 **Gold ES**, Diercks AH, Podolsky I, Podyminogin RL, Askovich PS, Treuting PM, Aderem A. 25-Hydroxycholesterol acts as an amplifier of inflammatory signaling. *Proc Natl Acad Sci U S A* 2014; **111**: 10666-10671 [PMID: 24994901 DOI: 10.1073/pnas.1404271111]

80 **Yu G**, Sun W, Wang W, Le C, Liang D, Shuai L. Overexpression of microRNA-202-3p in bone marrow mesenchymal stem cells improves cerebral ischemia-reperfusion injury by promoting angiogenesis and inhibiting inflammation. *Aging (Albany NY)* 2021; **13**: 11877-11888 [PMID: 33893248 DOI: 10.18632/aging.202889]

81 **Deng M**, Liu J, He J, Lan Z, Cheng S, Hu Z, Xiao H. Bone marrow-derived mesenchymal stem cells overexpressed with miR-182-5p protects against brain injury in a mouse model of cerebral ischemia. *J Stroke Cerebrovasc Dis* 2022; **31**: 106748 [PMID: 36087376 DOI: 10.1016/j.jstrokecerebrovasdis.2022.106748]

82 **Li X**, Liu L, Yang J, Yu Y, Chai J, Wang L, Ma L, Yin H. Exosome Derived From Human Umbilical Cord Mesenchymal Stem Cell Mediates MiR-181c Attenuating Burn-induced Excessive Inflammation. *EBioMedicine* 2016; **8**: 72-82 [PMID: 27428420 DOI: 10.1016/j.ebiom.2016.04.030]

83 **Haupt M**, Zheng X, Kuang Y, Lieschke S, Janssen L, Bosche B, Jin F, Hein K, Kilic E, Venkataramani V, Hermann DM, Bähr M, Doeppner TR. Lithium modulates miR-1906 levels of mesenchymal stem cell-derived extracellular vesicles contributing to poststroke neuroprotection by toll-like receptor 4 regulation. *Stem Cells Transl Med* 2021; **10**: 357-373 [PMID: 33146943 DOI: 10.1002/sctm.20-0086]

84 **Liu W**, Rong Y, Wang J, Zhou Z, Ge X, Ji C, Jiang D, Gong F, Li L, Chen J, Zhao S, Kong F, Gu C, Fan J, Cai W. Exosome-shuttled miR-216a-5p from hypoxic preconditioned mesenchymal stem cells repair traumatic spinal cord injury by shifting microglial M1/M2 polarization. *J Neuroinflammation* 2020; **17**: 47 [PMID: 32019561 DOI: 10.1186/s12974-020-1726-7]

85 **Zhang Z**, Zou X, Zhang R, Xie Y, Feng Z, Li F, Han J, Sun H, Ouyang Q, Hua S, Lv B, Hua T, Liu Z, Cai Y, Zou Y, Tang Y, Jiang X. Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation *via* suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. *Aging (Albany NY)* 2021; **13**: 3060-3079 [PMID: 33479185 DOI: 10.18632/aging.202466]

86 **Wang Z**, Liu F, Wei M, Qiu Y, Ma C, Shen L, Huang Y. Chronic constriction injury-induced microRNA-146a-5p alleviates neuropathic pain through suppression of IRAK1/TRAF6 signaling pathway. *J Neuroinflammation* 2018; **15**: 179 [PMID: 29885668 DOI: 10.1186/s12974-018-1215-4]

87 **Zhao Y**, Gan Y, Xu G, Hua K, Liu D. Exosomes from MSCs overexpressing microRNA-223-3p attenuate cerebral ischemia through inhibiting microglial M1 polarization mediated inflammation. *Life Sci* 2020; **260**: 118403 [PMID: 32926923 DOI: 10.1016/j.lfs.2020.118403]

88 **Wang Y**, Yang Y, Zhang S, Li C, Zhang L. Modulation of neuroinflammation by cysteinyl leukotriene 1 and 2 receptors: implications for cerebral ischemia and neurodegenerative diseases. *Neurobiol Aging* 2020; **87**: 1-10 [PMID: 31986345 DOI: 10.1016/j.neurobiolaging.2019.12.013]

89 **Zhao Y**, Gan Y, Xu G, Yin G, Liu D. MSCs-Derived Exosomes Attenuate Acute Brain Injury and Inhibit Microglial Inflammation by Reversing CysLT2R-ERK1/2 Mediated Microglia M1 Polarization. *Neurochem Res* 2020; **45**: 1180-1190 [PMID: 32112178 DOI: 10.1007/s11064-020-02998-0]

90 **Morales L**, Oliveros JC, Enjuanes L, Sola I. Contribution of Host miRNA-223-3p to SARS-CoV-Induced Lung Inflammatory Pathology. *mBio* 2022; **13**: e0313521 [PMID: 35229638 DOI: 10.1128/mbio.03135-21]

91 **Jiang M**, Wang H, Jin M, Yang X, Ji H, Jiang Y, Zhang H, Wu F, Wu G, Lai X, Cai L, Hu R, Xu L, Li L. Exosomes from MiR-30d-5p-ADSCs Reverse Acute Ischemic Stroke-Induced, Autophagy-Mediated Brain Injury by Promoting M2 Microglial/Macrophage Polarization. *Cell Physiol Biochem* 2018; **47**: 864-878 [PMID: 29807362 DOI: 10.1159/000490078]

92 **Hu K**, Gao Y, Chu S, Chen N. Review of the effects and Mechanisms of microglial autophagy in ischemic stroke. *Int Immunopharmacol* 2022; **108**: 108761 [PMID: 35729827 DOI: 10.1016/j.intimp.2022.108761]

93 **Kang C**, Avery L. To be or not to be, the level of autophagy is the question: dual roles of autophagy in the survival response to starvation. *Autophagy* 2008; **4**: 82-84 [PMID: 17952023 DOI: 10.4161/auto.5154]

94 **Zang J**, Wu Y, Su X, Zhang T, Tang X, Ma D, Li Y, Liu Y, Weng Z, Liu X, Tsang CK, Xu A, Lu D. Inhibition of PDE1-B by Vinpocetine Regulates Microglial Exosomes and Polarization Through Enhancing Autophagic Flux for Neuroprotection Against Ischemic Stroke. *Front Cell Dev Biol* 2020; **8**: 616590 [PMID: 33614626 DOI: 10.3389/fcell.2020.616590]

95 **Kulkarni A**, Dong A, Kulkarni VV, Chen J, Laxton O, Anand A, Maday S. Differential regulation of autophagy during metabolic stress in astrocytes and neurons. *Autophagy* 2020; **16**: 1651-1667 [PMID: 31876243 DOI: 10.1080/15548627.2019.1703354]

96 **Pei X**, Li Y, Zhu L, Zhou Z. Astrocyte-derived exosomes transfer miR-190b to inhibit oxygen and glucose deprivation-induced autophagy and neuronal apoptosis. *Cell Cycle* 2020; **19**: 906-917 [PMID: 32150490 DOI: 10.1080/15384101.2020.1731649]

97 **Cheng C**, Chen X, Wang Y, Cheng W, Zuo X, Tang W, Huang W. MSCsderived exosomes attenuate ischemia-reperfusion brain injury and inhibit microglia apoptosis might *via* exosomal miR-26a-5p mediated suppression of CDK6. *Mol Med* 2021; **27**: 67 [PMID: 34215174 DOI: 10.1186/s10020-021-00324-0]

98 **Zhao H**, Wang J, Gao L, Wang R, Liu X, Gao Z, Tao Z, Xu C, Song J, Ji X, Luo Y. MiRNA-424 protects against permanent focal cerebral ischemia injury in mice involving suppressing microglia activation. *Stroke* 2013; **44**: 1706-1713 [PMID: 23613494 DOI: 10.1161/STROKEAHA.111.000504]

99 **Liu X**, Zhang M, Liu H, Zhu R, He H, Zhou Y, Zhang Y, Li C, Liang D, Zeng Q, Huang G. Bone marrow mesenchymal stem cell-derived exosomes attenuate cerebral ischemia-reperfusion injury-induced neuroinflammation and pyroptosis by modulating microglia M1/M2 phenotypes. *Exp Neurol* 2021; **341**: 113700 [PMID: 33741350 DOI: 10.1016/j.expneurol.2021.113700]

100 **Zong L**, Huang P, Song Q, Kang Y. Bone marrow mesenchymal stem cells-secreted exosomal H19 modulates lipopolysaccharides-stimulated microglial M1/M2 polarization and alleviates inflammation-mediated neurotoxicity. *Am J Transl Res* 2021; **13**: 935-951 [PMID: 33841631]

101 **Hou K**, Li G, Zhao J, Xu B, Zhang Y, Yu J, Xu K. Bone mesenchymal stem cell-derived exosomal microRNA-29b-3p prevents hypoxic-ischemic injury in rat brain by activating the PTEN-mediated Akt signaling pathway. *J Neuroinflammation* 2020; **17**: 46 [PMID: 32014002 DOI: 10.1186/s12974-020-1725-8]

102 **Wang R**, Zhang X, Zhang J, Fan Y, Shen Y, Hu W, Chen Z. Oxygen-glucose deprivation induced glial scar-like change in astrocytes. *PLoS One* 2012; **7**: e37574 [PMID: 22629422 DOI: 10.1371/journal.pone.0037574]

103 **Saijo K**, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* 2009; **137**: 47-59 [PMID: 19345186 DOI: 10.1016/j.cell.2009.01.038]

104 **Xian P**, Hei Y, Wang R, Wang T, Yang J, Li J, Di Z, Liu Z, Baskys A, Liu W, Wu S, Long Q. Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice. *Theranostics* 2019; **9**: 5956-5975 [PMID: 31534531 DOI: 10.7150/thno.33872]

105 **Liu K**, Cai GL, Zhuang Z, Pei SY, Xu SN, Wang YN, Wang H, Wang X, Cui C, Sun MC, Guo SH, Jia KP, Wang XZ, Cai GF. Interleukin-1β-Treated Mesenchymal Stem Cells Inhibit Inflammation in Hippocampal Astrocytes Through Exosome-Activated Nrf-2 Signaling. *Int J Nanomedicine* 2021; **16**: 1423-1434 [PMID: 33654394 DOI: 10.2147/IJN.S289914]

106 **Wang L**, Pei S, Han L, Guo B, Li Y, Duan R, Yao Y, Xue B, Chen X, Jia Y. Mesenchymal Stem Cell-Derived Exosomes Reduce A1 Astrocytes *via* Downregulation of Phosphorylated NFκB P65 Subunit in Spinal Cord Injury. *Cell Physiol Biochem* 2018; **50**: 1535-1559 [PMID: 30376671 DOI: 10.1159/000494652]

107 **Sivandzade F**, Prasad S, Bhalerao A, Cucullo L. NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. *Redox Biol* 2019; **21**: 101059 [PMID: 30576920 DOI: 10.1016/j.redox.2018.11.017]

108 **Lai X**, Wang Y, Wang X, Liu B, Rong L. miR-146a-5p-modified hUCMSC-derived exosomes facilitate spinal cord function recovery by targeting neurotoxic astrocytes. *Stem Cell Res Ther* 2022; **13**: 487 [PMID: 36175984 DOI: 10.1186/s13287-022-03116-3]

109 **Bi F**, Huang C, Tong J, Qiu G, Huang B, Wu Q, Li F, Xu Z, Bowser R, Xia XG, Zhou H. Reactive astrocytes secrete lcn2 to promote neuron death. *Proc Natl Acad Sci U S A* 2013; **110**: 4069-4074 [PMID: 23431168 DOI: 10.1073/pnas.1218497110]

110 **Hochmeister S**, Engel O, Adzemovic MZ, Pekar T, Kendlbacher P, Zeitelhofer M, Haindl M, Meisel A, Fazekas F, Seifert-Held T. Lipocalin-2 as an Infection-Related Biomarker to Predict Clinical Outcome in Ischemic Stroke. *PLoS One* 2016; **11**: e0154797 [PMID: 27152948 DOI: 10.1371/journal.pone.0154797]

111 **Deng Y**, Chen D, Gao F, Lv H, Zhang G, Sun X, Liu L, Mo D, Ma N, Song L, Huo X, Yan T, Zhang J, Miao Z. Exosomes derived from microRNA-138-5p-overexpressing bone marrow-derived mesenchymal stem cells confer neuroprotection to astrocytes following ischemic stroke *via* inhibition of LCN2. *J Biol Eng* 2019; **13**: 71 [PMID:

31485266 DOI: 10.1186/s13036-019-0193-0]
112 **Donega V**, Nijboer CH, van Tilborg G, Dijkhuizen RM, Kavelaars A, Heijnen CJ. Intranasally administered mesenchymal stem cells promote a regenerative niche for repair of neonatal ischemic brain injury. *Exp Neurol* 2014; **261**: 53-64 [PMID: 24945601 DOI: 10.1016/j.expneurol.2014.06.009]
113 **Zhu LH**, Bai X, Zhang N, Wang SY, Li W, Jiang L. Improvement of human umbilical cord mesenchymal stem cell transplantation on glial cell and behavioral function in a neonatal model of periventricular white matter damage. *Brain Res* 2014; **1563**: 13-21 [PMID: 24680746 DOI: 10.1016/j.brainres.2014.03.030]
114 **Huang W**, Lv B, Zeng H, Shi D, Liu Y, Chen F, Li F, Liu X, Zhu R, Yu L, Jiang X. Paracrine Factors Secreted by MSCs Promote Astrocyte Survival Associated With GFAP Downregulation After Ischemic Stroke *via* p38 MAPK and JNK. *J Cell Physiol* 2015; **230**: 2461-2475 [PMID: 25752945 DOI: 10.1002/jcp.24981]
115 **Li Z**, Song Y, He T, Wen R, Li Y, Chen T, Huang S, Wang Y, Tang Y, Shen F, Tian HL, Yang GY, Zhang Z. M2 microglial small extracellular vesicles reduce glial scar formation *via* the miR-124/STAT3 pathway after ischemic stroke in mice. *Theranostics* 2021; **11**: 1232-1248 [PMID: 33391532 DOI: 10.7150/thno.48761]
116 **Peng Z**, Lu H, Yang Q, Xie Q. Astrocyte Reprogramming in Stroke: Opportunities and Challenges. *Front Aging Neurosci* 2022; **14**: 885707 [PMID: 35663583 DOI: 10.3389/fnagi.2022.885707]
117 **Ge LJ**, Yang FH, Li W, Wang T, Lin Y, Feng J, Chen NH, Jiang M, Wang JH, Hu XT, Chen G. In vivo Neuroregeneration to Treat Ischemic Stroke Through NeuroD1 AAV-Based Gene Therapy in Adult Non-human Primates. *Front Cell Dev Biol* 2020; **8**: 590008 [PMID: 33224952 DOI: 10.3389/fcell.2020.590008]
118 **Xin H**, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, Shang X, Zhang ZG, Chopp M. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells* 2012; **30**: 1556-1564 [PMID: 22605481 DOI: 10.1002/stem.1129]
119 **Xin H**, Li Y, Liu Z, Wang X, Shang X, Cui Y, Zhang ZG, Chopp M. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats *via* transfer of exosome-enriched extracellular particles. *Stem Cells* 2013; **31**: 2737-2746 [PMID: 23630198 DOI: 10.1002/stem.1409]
120 **Yao J**, Zheng J, Cai J, Zeng K, Zhou C, Zhang J, Li S, Li H, Chen L, He L, Chen H, Fu H, Zhang Q, Chen G, Yang Y, Zhang Y. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response. *FASEB J* 2019; **33**: 1695-1710 [PMID: 30226809 DOI: 10.1096/fj.201800131RR]
121 **Wang C**, Börger V, Sardari M, Murke F, Skuljec J, Pul R, Hagemann N, Dzyubenko E, Dittrich R, Gregorius J, Hasenberg M, Kleinschnitz C, Popa-Wagner A, Doeppner TR, Gunzer M, Giebel B, Hermann DM. Mesenchymal Stromal Cell-Derived Small Extracellular Vesicles Induce Ischemic Neuroprotection by Modulating Leukocytes and Specifically Neutrophils. *Stroke* 2020; **51**: 1825-1834 [PMID: 32312217 DOI: 10.1161/STROKEAHA.119.028012]
122 **Mahmoudi M**, Taghavi-Farahabadi M, Rezaei N, Hashemi SM. Comparison of the effects of adipose tissue mesenchymal stromal cell-derived exosomes with conditioned media on neutrophil function and apoptosis. *Int Immunopharmacol* 2019; **74**: 105689 [PMID: 31207404 DOI: 10.1016/j.intimp.2019.105689]
123 **Taghavi-Farahabadi M**, Mahmoudi M, Rezaei N, Hashemi SM. Wharton's Jelly Mesenchymal Stem Cells Exosomes and Conditioned Media Increased Neutrophil Lifespan and Phagocytosis Capacity. *Immunol Invest* 2021; **50**: 1042-1057 [PMID: 32777963 DOI: 10.1080/08820139.2020.1801720]
124 **Loh JT**, Zhang B, Teo JKH, Lai RC, Choo ABH, Lam KP, Lim SK. Mechanism for the attenuation of neutrophil and complement hyperactivity by MSC exosomes. *Cytotherapy* 2022; **24**: 711-719 [PMID: 35177337 DOI: 10.1016/j.jcyt.2021.12.003]
125 **Lu T**, Zhang J, Cai J, Xiao J, Sui X, Yuan X, Li R, Li Y, Yao J, Lv G, Chen X, Chen H, Zeng K, Liu Y, Chen W, Chen G, Yang Y, Zheng J, Zhang Y. Extracellular vesicles derived from mesenchymal stromal cells as nanotherapeutics for liver ischaemia-reperfusion injury by transferring mitochondria to modulate the formation of neutrophil extracellular traps. *Biomaterials* 2022; **284**: 121486 [PMID: 35447404 DOI: 10.1016/j.biomaterials.2022.121486]
126 **Krausgruber T**, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, Udalova IA. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 2011; **12**: 231-238 [PMID: 21240265 DOI: 10.1038/ni.1990]
127 **Fang H**, Yang M, Pan Q, Jin HL, Li HF, Wang RR, Wang QY, Zhang JP. MicroRNA-22-3p alleviates spinal cord ischemia/reperfusion injury by modulating M2 macrophage polarization *via* IRF5. *J Neurochem* 2021; **156**: 106-120 [PMID: 32406529 DOI: 10.1111/jnc.15042]
128 **Chang Q**, Hao Y, Wang Y, Zhou Y, Zhuo H, Zhao G. Bone marrow mesenchymal stem cell-derived exosomal microRNA-125a promotes M2 macrophage polarization in spinal cord injury by downregulating IRF5. *Brain Res Bull* 2021; **170**: 199-210 [PMID: 33609602 DOI: 10.1016/j.brainresbull.2021.02.015]
129 **Heo JS**, Choi Y, Kim HO. Adipose-Derived Mesenchymal Stem Cells Promote M2 Macrophage Phenotype through Exosomes. *Stem Cells Int* 2019; **2019**: 7921760 [PMID: 31781246 DOI: 10.1155/2019/7921760]
130 **Heo JS**, Kim S. Human adipose mesenchymal stem cells modulate inflammation and angiogenesis through exosomes. *Sci Rep* 2022; **12**: 2776 [PMID: 35177768 DOI: 10.1038/s41598-022-06824-1]
131 **Das A**, Ganesh K, Khanna S, Sen CK, Roy S. Engulfment of apoptotic cells by macrophages: a role of microRNA-21 in the resolution of wound inflammation. *J Immunol* 2014; **192**: 1120-1129 [PMID: 24391209 DOI: 10.4049/jimmunol.1300613]
132 **Wu H**, Fan H, Shou Z, Xu M, Chen Q, Ai C, Dong Y, Liu Y, Nan Z, Wang Y, Yu T, Liu X. Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. *Int Immunopharmacol* 2019; **68**: 204-212 [PMID: 30654310 DOI: 10.1016/j.intimp.2018.12.043]
133 **Hsu LW**, Huang KT, Nakano T, Chiu KW, Chen KD, Goto S, Chen CL. MicroRNA-301a inhibition enhances the immunomodulatory functions of adipose-derived mesenchymal stem cells by induction of macrophage M2 polarization. *Int J Immunopathol Pharmacol* 2020; **34**: 2058738420966092 [PMID: 33121303 DOI: 10.1177/2058738420966092]
134 **Becker K**, Kindrick D, Relton J, Harlan J, Winn R. Antibody to the alpha4 integrin decreases infarct size in transient focal

cerebral ischemia in rats. *Stroke* 2001; **32**: 206-211 [PMID: 11136938 DOI: 10.1161/01.str.32.1.206]

135 **Blazquez R**, Sanchez-Margallo FM, de la Rosa O, Dalemans W, Alvarez V, Tarazona R, Casado JG. Immunomodulatory Potential of Human Adipose Mesenchymal Stem Cells Derived Exosomes on in vitro Stimulated T Cells. *Front Immunol* 2014; **5**: 556 [PMID: 25414703 DOI: 10.3389/fimmu.2014.00556]

136 **Dabrowska S**, Andrzejewska A, Strzemecki D, Muraca M, Janowski M, Lukomska B. Human bone marrow mesenchymal stem cell-derived extracellular vesicles attenuate neuroinflammation evoked by focal brain injury in rats. *J Neuroinflammation* 2019; **16**: 216 [PMID: 31722731 DOI: 10.1186/s12974-019-1602-5]

137 **Du YM**, Zhuansun YX, Chen R, Lin L, Lin Y, Li JG. Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma. *Exp Cell Res* 2018; **363**: 114-120 [PMID: 29277503 DOI: 10.1016/j.yexcr.2017.12.021]

138 **Liu J**, Cao X. Regulatory dendritic cells in autoimmunity: A comprehensive review. *J Autoimmun* 2015; **63**: 1-12 [PMID: 26255250 DOI: 10.1016/j.jaut.2015.07.011]

139 **Liu J**, Liu Q, Chen X. The Immunomodulatory Effects of Mesenchymal Stem Cells on Regulatory B Cells. *Front Immunol* 2020; **11**: 1843 [PMID: 32922398 DOI: 10.3389/fimmu.2020.01843]

140 **Yang H**, Chen J. Bone marrow mesenchymal stem cell-derived exosomes carrying long noncoding RNA ZFAS1 alleviate oxidative stress and inflammation in ischemic stroke by inhibiting microRNA-15a-5p. *Metab Brain Dis* 2022; **37**: 2545-2557 [PMID: 35907132 DOI: 10.1007/s11011-022-00997-4]

141 **Li C**, Fei K, Tian F, Gao C, Yang S. Adipose-derived mesenchymal stem cells attenuate ischemic brain injuries in rats by modulating miR-21-3p/MAT2B signaling transduction. *Croat Med J* 2019; **60**: 439-448 [PMID: 31686458 DOI: 10.3325/cmj.2019.60.439]

142 **Shahir M**, Mahmoud Hashemi S, Asadirad A, Varahram M, Kazempour-Dizaji M, Folkerts G, Garssen J, Adcock I, Mortaz E. Effect of mesenchymal stem cell-derived exosomes on the induction of mouse tolerogenic dendritic cells. *J Cell Physiol* 2020; **235**: 7043-7055 [PMID: 32043593 DOI: 10.1002/jcp.29601]

143 **Dong C**, Chen M, Cai B, Zhang C, Xiao G, Luo W. Mesenchymal Stem Cell-Derived Exosomes Improved Cerebral Infarction *via* Transferring miR-23a-3p to Activate Microglia. *Neuromolecular Med* 2022; **24**: 290-298 [PMID: 35001328 DOI: 10.1007/s12017-021-08686-8]

144 **Gong Z**, Pan J, Shen Q, Li M, Peng Y. Mitochondrial dysfunction induces NLRP3 inflammasome activation during cerebral ischemia/reperfusion injury. *J Neuroinflammation* 2018; **15**: 242 [PMID: 30153825 DOI: 10.1186/s12974-018-1282-6]

145 **Zeng Q**, Zhou Y, Liang D, He H, Liu X, Zhu R, Zhang M, Luo X, Wang Y, Huang G. Exosomes Secreted From Bone Marrow Mesenchymal Stem Cells Attenuate Oxygen-Glucose Deprivation/Reoxygenation-Induced Pyroptosis in PC12 Cells by Promoting AMPK-Dependent Autophagic Flux. *Front Cell Neurosci* 2020; **14**: 182 [PMID: 32765221 DOI: 10.3389/fncel.2020.00182]

146 **Tian T**, Zhang HX, He CP, Fan S, Zhu YL, Qi C, Huang NP, Xiao ZD, Lu ZH, Tannous BA, Gao J. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* 2018; **150**: 137-149 [PMID: 29040874 DOI: 10.1016/j.biomaterials.2017.10.012]

147 **Yari H**, Mikhailova MV, Mardasi M, Jafarzadehgharehziaaddin M, Shahrokh S, Thangavelu L, Ahmadi H, Shomali N, Yaghoubi Y, Zamani M, Akbari M, Alesaeidi S. Emerging role of mesenchymal stromal cells (MSCs)-derived exosome in neurodegeneration-associated conditions: a groundbreaking cell-free approach. *Stem Cell Res Ther* 2022; **13**: 423 [PMID: 35986375 DOI: 10.1186/s13287-022-03122-5]

148 **Zhang H**, Wu J, Fan Q, Zhou J, Liu S, Zang J, Ye J, Xiao M, Tian T, Gao J. Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice. *J Nanobiotechnology* 2019; **17**: 29 [PMID: 30782171 DOI: 10.1186/s12951-019-0461-7]

149 **Yang J**, Zhang X, Chen X, Wang L, Yang G. Exosome Mediated Delivery of miR-124 Promotes Neurogenesis after Ischemia. *Mol Ther Nucleic Acids* 2017; **7**: 278-287 [PMID: 28624203 DOI: 10.1016/j.omtn.2017.04.010]

150 **Kim M**, Kim G, Hwang DW, Lee M. Delivery of High Mobility Group Box-1 siRNA Using Brain-Targeting Exosomes for Ischemic Stroke Therapy. *J Biomed Nanotechnol* 2019; **15**: 2401-2412 [PMID: 31748020 DOI: 10.1166/jbn.2019.2866]

151 **Phan J**, Kumar P, Hao D, Gao K, Farmer D, Wang A. Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy. *J Extracell Vesicles* 2018; **7**: 1522236 [PMID: 30275938 DOI: 10.1080/20013078.2018.1522236]

152 **Rafiq QA**, Coopman K, Nienow AW, Hewitt CJ. Systematic microcarrier screening and agitated culture conditions improves human mesenchymal stem cell yield in bioreactors. *Biotechnol J* 2016; **11**: 473-486 [PMID: 26632496 DOI: 10.1002/biot.201400862]

153 **Yan IK**, Shukla N, Borrelli DA, Patel T. Use of a Hollow Fiber Bioreactor to Collect Extracellular Vesicles from Cells in Culture. *Methods Mol Biol* 2018; **1740**: 35-41 [PMID: 29388134 DOI: 10.1007/978-1-4939-7652-2_4]

154 **Wang J**, Bonacquisti EE, Brown AD, Nguyen J. Boosting the Biogenesis and Secretion of Mesenchymal Stem Cell-Derived Exosomes. *Cells* 2020; **9** [PMID: 32182815 DOI: 10.3390/cells9030660]

155 **NIH**. Shahid Beheshti University of Medical Sciences. clinicaltrials.gov; 2021. [cited 7 November 2022]. Available from: https://clinicaltrials.gov/ct2/show/NCT03384433

156 **Darsalia V**, Allison SJ, Cusulin C, Monni E, Kuzdas D, Kallur T, Lindvall O, Kokaia Z. Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. *J Cereb Blood Flow Metab* 2011; **31**: 235-242 [PMID: 20531461 DOI: 10.1038/jcbfm.2010.81]

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MINIREVIEWS

Disease modeling of desmosome-related cardiomyopathy using induced pluripotent stem cell-derived cardiomyocytes

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Abstract

Cardiomyopathy is a pathological condition characterized by cardiac pump failure due to myocardial dysfunction and the major cause of advanced heart failure requiring heart transplantation. Although optimized medical therapies have been developed for heart failure during the last few decades, some patients with cardiomyopathy exhibit advanced heart failure and are refractory to medical therapies. Desmosome, which is a dynamic cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause arrhythmogenic cardiomyopathy (AC), a rare inheritable disease, and predispose patients to sudden cardiac death and heart failure. Recent advances in sequencing technologies have elucidated the genetic basis of cardiomyopathies and revealed that desmosome-related cardiomyopathy is concealed in broad cardiomyopathies. Among desmosomal genes, mutations in *PKP2* (which encodes PKP2) are most frequently identified in patients with AC. *PKP2* deficiency causes various pathological cardiac phenotypes. Human cardiomyocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows the precise arrangement of the targeted genome, are powerful experimental tools for studying disease. This review summarizes the current issues associated with practical medicine for advanced heart failure and the recent advances in disease modeling using iPSC-derived cardiomyocytes targeting desmosome-related cardiomyopathy caused by *PKP2* deficiency.

Key Words: Cardiomyopathy; Advanced heart failure; Induced pluripotent stem cell-derived cardiomyocytes; Desmosome; Genome editing; Gene therapy

Core Tip: Prevention of advanced heart failure caused by cardiomyopathy is an urgent unmet need in the field of cardiovascular medicine. Desmosome, a cell-to-cell junctional component, maintains the structural integrity of heart tissues. Genetic mutations in desmosomal genes cause desmosome-related cardiomyopathy, an intractable disease refractory to standard medical therapies. This review introduces the recent advances in disease modeling of desmosome-related cardiomyopathy caused by *PKP2* mutations using induced pluripotent stem cell-derived cardiomyocytes.

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INTRODUCTION

Heart failure is a clinical syndrome characterized by dyspnea, malaise, swelling, and/or decreased exercise capacity owing to impaired cardiac pumping function[1]. The established optimal medical therapies for heart failure have increased the survival rates of patients in the last few decades[2-4]. However, some patients are refractory to medical therapies and develop symptoms that are diagnosed as advanced heart failure. Currently, the therapeutic strategies available for these patients are heart transplantation and implantation of the ventricular assisting device[1,5]. Cardiomyopathy is a disease of cardiac pump failure due to myocardial dysfunction and is the major cause of advanced heart failure requiring heart transplantation[6-11]. Cardiomyopathies are differentially diagnosed mainly by using imaging modalities, including echocardiography, scintigraphy, computed tomography, magnetic resonance imaging, and cardiac catheterization. Based on the findings of these modalities, cardiomyopathies are classified into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), or other rare cardiomyopathies, such as arrhythmogenic right ventricular cardiomyopathy (ARVC)[12]. Among 36,883 heart transplantation recipients registered in the International Society for Heart and Lung Transplantation Thoracic Organ Transplant Registry between 2010 and 2018, the major primary diagnoses were non-ischemic DCM (50.8%), ischemic cardiomyopathy (ICM) (32.4%) with coronary artery disease, RCM (3.5%), and HCM (3.4%)[13]. In Japan, cardiomyopathies [DCM (64%), end-stage HCM with left ventricular systolic dysfunction (12%), and ICM (9%)] account for more than three-quarters of underlying diseases among heart transplant recipients[14]. ARVC, a rare inherited disease, is characterized by the risk of life-threatening arrhythmias, myocardial dysfunction, and fibrofatty replacement of myocardial tissue, predisposing the patients to sudden cardiac death and heart failure[9,11]. The prevalence of ARVC among the registrants for heart transplantation is rare (0.3% and 1%-2% in the United Network for Organ Sharing registry[15] and Japan Organ Transplant Network[14], respectively).

DESMOSOME-RELATED CARDIOMYOPATHY IS CONCEALED IN ADVANCED HEART FAILURE

Recent clinical studies utilizing high-throughput sequencing technologies have elucidated the genetic basis of cardiomyopathies, identified various causative genetic variants, and revealed the correlation between genetic factors and clinical phenotypes or cardiac morphologies in patients with cardiomyopathies[16-20]. ARVC is an inherited disease caused by mutations in desmosomal genes (*PKP2*, *JUP*, *DSC2*, *DSG2*, and *DSP*) (Figure 1)[11,21,22]. These genes encode the structural components of the desmosome, a dynamic junction between cells that maintain the structural integrity of heart tissues[23,24]. The original disease phenotypes of ARVC are characterized by predominant right ventricular enlargement and contractile dysfunction. However, recent studies have reported left ventricular or biventricular involvement in patients with ARVC, resulting in the use of a broad phrase [arrhythmogenic cardiomyopathy (AC)][9,11]. Although the prevalence of AC in patients with advanced heart failure is rare, recent genetic analyses in large cohorts have demonstrated an increased incidence of desmosomal gene mutations in patients with DCM[18,25,26], which is the most frequent basal disease among heart transplantation registrants. Furthermore, homozygosity and compound or digenic heterozygosity of desmosomal genes are not rare, and patients with combined mutations exhibit a severe phenotype[27-30]. Recently, we identified *DSG2*-deficient cardiomyopathy caused by a rare homozygous stop-gain mutation in a patient initially diagnosed with idiopathic sporadic DCM[30]. *Dsg2* deficiency is associated with embryonic lethality in mice. Additionally, *Dsg2*-depleted embryonic stem cells do not proliferate[31]. However, a human male patient with a complete lack of *DSG2*

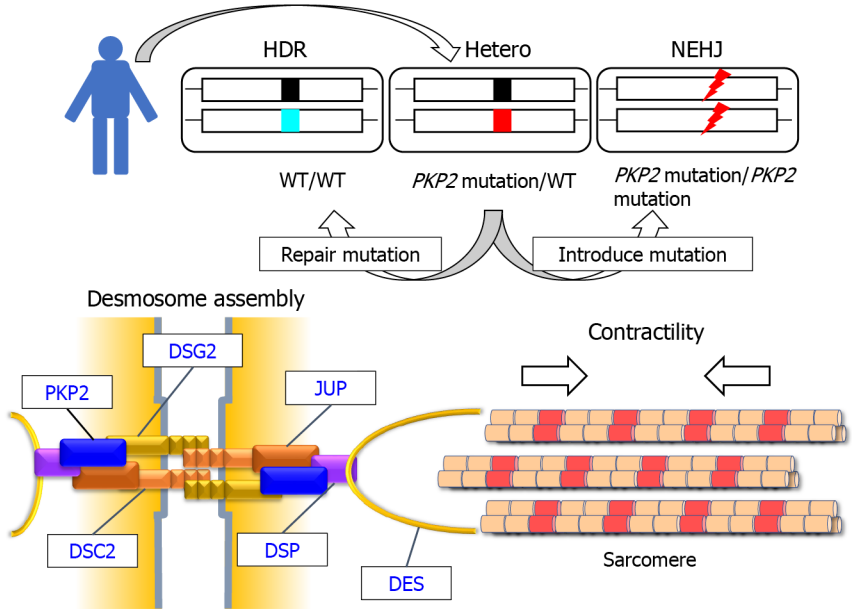

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Figure 1 Modeling impaired desmosome assembly and reduced contractility using isogenic induced pluripotent stem cell-derived cardiomyocytes with the precisely adjusted dose of *PKP2*. Heterozygous frameshift mutation in patient-derived induced pluripotent stem cells (iPSCs) was repaired through homology-directed repair. Homozygous frameshift mutations were introduced in *PKP2* through non-homologous end joining in patient-derived iPSCs. The generated isogenic iPSC-derived cardiomyocytes with the precisely adjusted expression of *PKP2* recapitulated impaired desmosome assembly and reduced contractility caused by *PKP2* deficiency. Desmosomal cadherin proteins (DSG2 and DSC2) form homo-dimers and hetero-dimers. PKP2 is a scaffold protein for desmosomal cadherins, JUP, and DSP. Desmosomes are linked to sarcomere structure *via* the intermediate filament protein DES that targets both desmosome and Z disc structure. HDR: Homology-directed repair; NEHJ: Non-homologous end joining; Hetero: Heterozygous mutation.

expression did not exhibit pathological phenotypes at birth but developed advanced heart failure during the teenage years. Immunohistochemical and transmission electron microscopy analyses of left ventricular heart tissues revealed that the loss of *DSG2* leads to aberrant deposition of desmosomal proteins and disruption of intercalated discs in cardiomyocytes. These findings suggest that desmosome-related cardiomyopathy is concealed in patients with advanced heart failure who are diagnosed with idiopathic DCM. As desmosome impairment is the most upstream molecular change in these patients, experimental studies must focus on elucidating the molecular mechanisms underlying the instability of cell-to-cell junctions to overcome advanced heart failure caused by desmosome-related cardiomyopathy. For disease modeling, patient-derived induced pluripotent stem cells (iPSCs) in combination with genome editing, which allows precise genomic modification of the targeted mutations, are powerful experimental tools to recapitulate pathological phenotypes based on the molecular factors of inherited cardiomyopathies[30,32-35].

PHENOTYPIC RECAPITULATION OF CARDIOMYOPATHY CAUSED BY PKP2 DEFICIENCY USING PATIENT-DERIVED IPSC-CMS

PKP2, which is encoded by *PKP2*, is a desmosomal protein localized to the outer dense plaque and functions as a scaffold for the other desmosome proteins DSG2, DSC2, JUP, and DSP[23,36] (Figure 1). Among the desmosomal genes, mutations in *PKP2* are most frequently identified in patients with AC [11,37-39], and have been extensively studied using patient-derived iPSC-CMs compared to other desmosomal genes (*DSG2*[30,40,41], *DSP*[42,43], and *DSC2*[44,45]). Various clinical phenotypes and pathological characteristics observed in patients with AC harboring *PKP2* mutations, downregulated desmosomal protein expression, upregulated lipogenesis, and increased apoptosis in heart tissues have been recapitulated using genetically engineered mouse models[11] and human cardiomyocytes differentiated from iPSCs[46-54] (Table 1). Most known mutations of *PKP2* are heterozygous and are missense, nonsense, and frameshift mutations. Studies on patient-derived iPSCs have identified that *PKP2* variants are heterozygous missense[48], heterozygous frameshift[46,47,49,50,54], homozygous frameshift[47,51], compound heterozygous, and frameshift[52] mutations. Disease-specific iPSCs are generated from fibroblasts[46-48,51], keratinocytes[49], adipose tissue-derived stromal cells[52], and peripheral blood mononuclear cells[54], whereas control iPSCs are generated from healthy subjects[46-49,51,52], human embryonic stem cells[50], or isogenic cells engineered from patient-derived iPSCs using genome editing[54]. Genome editing allows disease modeling by introducing heterozygous and

Table 1 Human disease model of *PKP2* deficiency using induced pluripotent stem cell-derived cardiomyocytes and experimental pathological phenotypes of arrhythmogenic cardiomyopathy

Genetic mutation	Origin of disease-specific iPSC	Experimental control	Desmosome proteins	Lipid accumulation	Apoptosis	Electrophysiology	Ultrastructure of desmosome	Contractility	Phenotypic rescue by gene replacement	Ref.
Heterozygous missense (c.1841T>C, p.L614P)	Dermal fibroblasts from a 30-yr-old male patient with AC	iPSCs from a 32-yr-old healthy male donor	Decreased JUP; No change in DSP, CDH2, and GJA1 (immunofluorescence staining at weeks 4-5)	Increased oil red O staining after exposure to adipogenic differentiation medium for 2 wk (oil red O staining)	NA	Ventricular-like action potential profile (single-cell patch-clamp recording (without control))	Increased cell width (TEM at weeks 4-5)	NA	NA	Ma *et al*[48]
Heterozygous frameshift (c.971_972ins, pA324fs335X); Heterozygous frameshift (c.148_151delACAG, p.T50SfsX110)	Dermal fibroblasts from a 30-yr-old male patient with AC	iPSCs from a healthy control	Decreased JUP and GJA1 (immunofluorescence staining)	Lipid droplet accumulation (TEM on day 40)	Increased apoptosis after serum starvation (TUNEL)	Prolonged field potential rise time (multielectrode array)	Widened and distorted desmosomes (TEM on day 40)	NA	NA	Caspi *et al* [46]
Homozygous frameshift (c.2484C>T leading to cryptic splicing); Heterozygous frameshift (c.2013delC, p.Lys672ArgfsX12)	Fibroblasts from a female patient with AC; Fibroblasts from a patient with AC	H9 human embryonic stem cell; iPSCs from cardiac fibroblasts of aborted fetus without a family history of AC	Nuclear translocation of JUP (immunofluorescence staining)	Increased lipogenesis after adipogenic stimulation for 4-5 wk (Nile red staining)	Increased apoptosis after adipogenic stimulation for 4-5 wk (TUNEL)	Slow intracellular calcium relaxation; Prolonged relaxation time (calcium imaging using Fura-2 acetoxymethyl on day 60)	NA	NA	NA	Kim *et al* [47]
Heterozygous frameshift (c.1760delT, p.V587Afsx655)	Dermal keratinocytes from a male patient with AC	iPSCs from dermal keratinocytes of a healthy control	Interrupted expression of DSP (immunofluorescence staining)	Lipid droplet accumulation after adipogenic stimulation for 4 wk (oil red O staining at months 3-4)	Genes associated with apoptosis remained unchanged (quantitative real-time PCR)	NA	NA	NA	NA	Dorn *et al* [49]
Homozygous frameshift (c.2484C>T leading to cryptic splicing)	Fibroblasts from a female patient with AC	iPSCs from a healthy control	Reduced JUP (immunofluorescence staining)	NA	NA	NA (decreased co-localization of NaV1.5 with PKP2)	NA	NA (increased pro-fibrotic gene expression after stretch)	NA	Martewicz *et al*[51]
Heterozygous frameshift (c.971_972InsT, p.A324fs335X)	A patient with AC	H9 human embryonic stem cells	Decreased membrane-localized JUP (immunofluorescence staining on day 34)	Increased lipid content (Nile red staining on day 34)	NA	Short action potential and slow spontaneous beat rate in engineered heart slices [optical mapping (relative to monolayer cardiomyocytes)]	NA	NA	NA	Blazeski *et al*[50]
Compound hetero-	Adipose tissue-	Gender-matched	Increased cytoplasmic and	No presence of	Not increased	Reduced sodium current	NA	NA	Restored	Khudiakov

zygous frameshift and missense (c.354delT, p.Y119MfsX23 and p.K859R)	derived mesenchymal multipotent stromal cells from a 14-yr-old female patient with AC	healthy donor	nuclear JUP levels (immunofluorescence staining on days 24-30)	lipid droplets (oil red O staining on day 24)	(PI staining at day 24-30)	density; Decreased action potential upstroke velocity (whole-cell patch-clamp and microelectrodes on days 24-30)			sodium current after lentiviral transduction of *PKP2*	*et al*[52]
Heterozygous and homozygous frameshift mutation (p.D109AfsX10, introduced mutation *via* genome editing)	Wild-type iPSC lines from two different donors with introduced heterozygous and homozygous frameshift mutations	Isogenic wild-type iPSCs	Decreased junctional localization of DSP and GJA1 (immunofluorescence staining); Impaired stability of junctional CDH2 (fluorescence recovery after photobleaching)	NA	NA	Prolonged action potential duration (optical voltage recording on day 30)	NA	Decreased systolic force (three-dimensional cardiac microtissues on day 40)	NA	Zhang *et al* [53]
Heterozygous frameshift mutation (c.1228dupG, p.D410fsX425)	Peripheral blood mononuclear cells from a female patient with AC	Isogenic iPSCs with corrected mutation (wild-type) and introduced homozygous frameshift mutations	Decreased area of desmosomes (DSG2, DSC2, and DSP) (immunofluorescence staining on day 14)	Lipid droplet accumulation in iPSC-CMs with homozygous frameshift mutations (TEM on day 28)	Increased apoptosis in iPSC-CMs with homozygous frameshift mutations (cleaved CASP3 expression on day 28)	Decreased propagation speed in iPSC-CMs with homozygous frameshift mutations (motion vector analysis on day 28)	Increased desmosome gap width (TEM on day 28)	Decreased contractility (contraction velocity and deformation distance evaluated using motion vector analysis on days 14 and 28)	Recovered contractility and desmosome assembly *via* AAV-mediated *PKP2* delivery	Inoue *et al* [54]

Gender of the patient or control donor is indicated if specified. Analytical methods along with time post-cardiomyocyte differentiation (if specified) are indicated. AAV: Adeno-associated virus; iPSC: Induced pluripotent stem cell; iPSC-CMs: Induced pluripotent stem cells-derived cardiomyocytes; PI: Propidium iodide; TEM: Transmission electron microscopy; NA: Not applicable; AC: Arrhythmogenic cardiomyopathy.

homozygous frameshift mutations in wild-type iPSC lines[53]. Decreased expression of desmosomal proteins, aberrant lipogenesis, and apoptosis of cardiomyocytes are observed in the heart tissues of patients with AC[9,55,56]. These pathological phenotypes are recapitulated in iPSC-CMs with *PKP2* mutations as determined using immunostaining[46-54], lipid staining[47-50], electron microscopy[46, 54], terminal transferase dUTP nick end labeling staining[46,47], and cleaved-CASP3 expression analysis [54]. Lethal arrhythmia is a hallmark of patients with AC. Arrhythmia phenotypes are recapitulated using iPSC-CMs with *PKP2* mutations as evidenced by the results of patch-clamp[48,52], multielectrode array[46], calcium imaging[47], and optical voltage recording[53]. In clinical settings, global or regional ventricular contractile dysfunction is defined as a major criterion for the diagnosis of ARVC in modified Task Force criteria[21] and Padua criteria[57]. However, the functional consequence in cardiomyocyte contractility caused by *PKP2* mutations has not been fully studied in human iPSC-CMs.

PKP2 DEFICIENCY AND CONTRACTILE DYSFUNCTION

We established iPSCs from a patient with AC harboring a heterozygous frameshift *PKP2* mutation

(c.1228dupG, p.D410fsX425) and generated an isogenic set of iPSC clones harboring three genotypes [heterozygous mutation (Hetero), homozygously corrected with homology-directed repair (HDR), and homozygously introduced frameshift mutations *via* non-homologous end joining (NHEJ)] using genome editing[54] (Figure 1). These isogenic sets of iPSCs comprise patient-derived Hetero-iPSCs, HDR-iPSCs with two-fold higher *PKP2* expression relative to Hetero-iPSCs, and NHEJ-iPSCs, which do not express *PKP2*, recapitulating both haploinsufficiency and complete loss of *PKP2*. After cardiomyocyte differentiation using the monolayer protocol with chemically defined medium[58], NHEJ-iPSC-CMs lacking *PKP2* expression exhibit lipid droplet accumulation, increased apoptosis, and decreased propagation rate (Table 1). However, patient-derived Hetero-iPSC-CMs with half-dose *PKP2* expression do not exhibit these pathological phenotypes, suggesting that the haploinsufficiency of *PKP2* is not sufficient to induce the above pathological phenotypes within 28 days after differentiation. In contrast, haploinsufficiency of *PKP2* decreased contractility, which was evaluated using motion vector analysis, within 14 days of differentiation. As the monolayer protocol confers strong contraction to iPSC-CMs on culture plates immediately after differentiation[58,59], continuous tensile overload may facilitate the contractile phenotype among isogenic iPSC-CMs. A recent study used isogenic iPSC-CMs in which heterozygous or homozygous frameshift mutation was introduced into wild-type iPSC-CMs[53]. The authors reported that *PKP2* deficiency decreased systolic force in three-dimensional cardiac microtissues. This further supported the functional relationship between *PKP2* deficiency and contractile dysfunction. An experimental study using cardiac tissue-specific *Pkp2* knockout mice demonstrated that the loss of *Pkp2* increased the distance between the cell periphery and DES, an intermediate filament protein in cardiomyocytes[60]. As DES connects Z-discs of sarcomeres to sarcolemmal costameres, desmosomes, and nuclear envelope[11,61], further experimental studies focusing on these cellular networks are required to elucidate the pathogenesis of desmosome-related cardiomyopathy.

DESMOSOME IMAGING USING THE ISOGENIC IPSC-CMS AND AAV-MEDIATED GENE REPLACEMENT

In the isogenic background, the haploinsufficiency of *PKP2* did not affect the localization or expression levels of desmosomal proteins in iPSC-CMs as evidenced by the results of immunostaining or western blotting analyses. However, the desmosome area represented by dot distribution on the cell periphery in Hetero-iPSC-CMs was significantly lower than that in HDR-iPSC-CMs[54], suggesting that desmosome assembly is impaired by *PKP2* haploinsufficiency. The impaired assembly of desmosomal proteins in human iPSC-CMs is supported by another study using isogenic iPSC-CMs. Fluorescence recovery after photobleaching experiments combined with lentivirus-mediated expression of fluorescent protein-tagged N-cadherin provided evidence that molecular stability of junctional N-cadherin is impaired by *PKP2* deficiency[53]. To trace the molecular behavior of endogenous proteins in cardiomyocytes, fluorescent tagging of the structural proteins through genome editing is a powerful tool[62,63]. However, fluorescent tagging of endogenous desmosomal genes might affect desmosome structures or cell-to-cell integrity in iPSCs or iPSC-CMs. We previously identified a patient with *DSG2*-deficient cardiomyopathy due to a rare homozygous stop-gain mutation and demonstrated that complete loss of *DSG2* in human iPSCs does not affect the differentiation or cellular morphology in iPSC-CMs[30]. These findings prompted us to use *DSG2* as the target of endogenous tagging by fluorescent protein to trace desmosome dynamics in live human iPSC-CMs. Genome editing targeting *DSG2* alleles was performed to establish the isogenic iPSC-CMs harboring identical two *DSG2* alleles comprising intact and knocked-in tdTomato alleles under the adjusted *PKP2* expression levels (Figure 2). The desmosome area (represented by desmoglein-2-tdTomato fusion protein) was significantly downregulated due to *PKP2* haploinsufficiency. Adeno-associated virus (AAV), a small, nonenveloped virus with a linear, single-stranded DNA, is widely used for gene therapy targeting human diseases, including heart failure[64,65]. AAV-mediated gene replacement of *PKP2* significantly restored the decreased contractility in Hetero-iPSC-CMs and NEHJ-iPSC-CMs, demonstrating the proof-of-concept for *PKP2* gene therapy in human cells. Furthermore, time-lapse imaging using NHEJ-iPSC-CMs captured the recovery of desmosomes, which gradually assembled at the cell periphery after AAV-mediated *PKP2* replacement (Figure 2). The established isogenic iPSCs harboring knocked-in tdTomato alleles allowed desmosome-imaging in living cells and provided distinct readouts for therapeutic development.

GENE REPLACEMENT THERAPY TARGETING HEART FAILURE

Several clinical trials using AAV-mediated gene replacement have been designed targeting cardiovascular disease[65,66]. A large-scale clinical trial was conducted as a randomized, multinational, double-blind, placebo-controlled phase 2 study targeting up to 250 patients with moderate-to-severe heart failure and reduced contractile function (CUPID2 trial)[67]. The study aimed to deliver

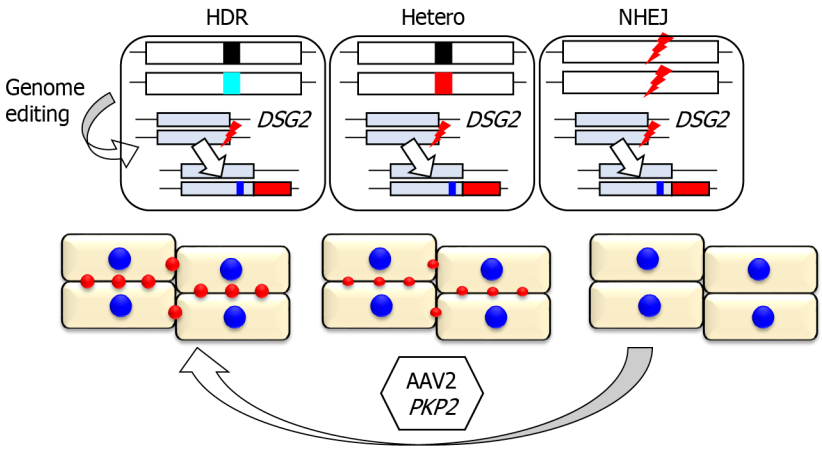

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Figure 2 Allele-specific fluorescent labeling of *DSG2* captures desmosome dynamics in isogenic induced pluripotent stem cell-derived cardiomyocytes. To establish a model for desmosome imaging, the tdTomato fluorescent reporter was knocked-in at the 3'-terminus of *DSG2* in the three established isogenic induced pluripotent stem cells (iPSCs) using genome editing. These isogenic iPSCs carried identical *DSG2* alleles comprising intact and knocked-in alleles distinguished by a synonymous single nucleotide variant (indicated as blue line). These iPSC-derived cardiomyocytes enable desmosome imaging and capturing desmosome recovery after adeno-associated virus-mediated replacement of *PKP2*. HDR: Homology-directed repair; NEHJ: Non-homologous end joining; AAV: Adeno-associated virus; Hetero: Heterozygous mutation.

sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) into heart tissues *via* intracoronary injection. SERCA2a regulates cardiomyocyte contraction and relaxation by transporting Ca^{2+} from the cytosol into the sarcoplasmic reticulum during diastole[68]. The deficiency of SERCA2a is associated with heart failure progression[69,70]. Although promising results were achieved in preceding preclinical and clinical studies[71-73], gene replacement of SERCA2a did not improve the clinical course of patients with heart failure[74]. The two clinical trials of gene therapy targeting patients with heart failure conducted in the same period (AGENT-HF[75] and SERCA-LVAD[76]) were terminated due to the neutral result of the CUPID2 trial and the lack of functional benefit. The amount of vector DNA in heart tissues obtained from patients who received gene therapy and subsequently underwent heart transplantation or mechanical circulatory support device implantation was low, suggesting that only a small proportion of cardiomyocytes expressed AAV-delivered SERCA2a in the myocardium. Although these clinical trials demonstrate the difficulty of gene delivery targeting human heart tissues, they provide the evidence for the safety of cardiac gene therapy and a basis for the design of future gene therapy trials. Recent genetic analysis clarified a large number of genetic mutations that cause cardiomyopathies with advanced heart failure in a loss-of-function manner and can be targeted by specific gene replacement therapy[77,78]. In desmosome-related cardiomyopathy, most of the identified mutations in *PKP2* are heterozygous[22,37,79,80]. However, in extremely rare cases, homozygous mutations of *PKP2* cause lethal infantile heart failure with left ventricular non-compaction or hypoplastic left heart syndrome[81-83]. No effective therapies are available for these patients who require a novel therapeutic approach for desmosome-related cardiomyopathy. Proof-of-concept studies for structural and functional recovery using both human iPSC-CM models and *in vivo* models are required for future clinical application.

CONCLUSION

Although human iPSC-CMs are immature and do not fully recapitulate *in vivo* heart tissues[59], tissue engineering approaches[84,85] will promote the maturation of iPSC-CMs and provide a useful tool in combination with genome editing. The isogenic iPSC-CMs that we established represent a human disease model that recapitulates reduced contractility and impaired desmosome assembly and provides a convenient cellular platform for therapeutic screening to examine upstream molecular targets of desmosome-related cardiomyopathy.

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FOOTNOTES

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REFERENCES

1 **Tsutsui H**, Ide T, Ito H, Kihara Y, Kinugawa K, Kinugawa S, Makaya M, Murohara T, Node K, Saito Y, Sakata Y, Shimizu W, Yamamoto K, Bando Y, Iwasaki YK, Kinugasa Y, Mizote I, Nakagawa H, Oishi S, Okada A, Tanaka A, Akasaka T, Ono M, Kimura T, Kosaka S, Kosuge M, Momomura SI. JCS/JHFS 2021 Guideline Focused Update on Diagnosis and Treatment of Acute and Chronic Heart Failure. *J Card Fail* 2021; **27**: 1404-1444 [PMID: 34600838 DOI: 10.1016/j.cardfail.2021.04.023]

2 **Levy D**, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, Murabito JM, Vasan RS. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002; **347**: 1397-1402 [PMID: 12409541 DOI: 10.1056/NEJMoa020265]

3 **Roger VL**, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. *JAMA* 2004; **292**: 344-350 [PMID: 15265849 DOI: 10.1001/jama.292.3.344]

4 **Jones NR**, Roalfe AK, Adoki I, Hobbs FDR, Taylor CJ. Survival of patients with chronic heart failure in the community: a systematic review and meta-analysis. *Eur J Heart Fail* 2019; **21**: 1306-1325 [PMID: 31523902 DOI: 10.1002/ejhf.1594]

5 **Ono M**, Yamaguchi O, Ohtani T, Kinugawa K, Saiki Y, Sawa Y, Shiose A, Tsutsui H, Fukushima N, Matsumiya G, Yanase M, Yamazaki K, Yamamoto K, Akiyama M, Imamura T, Iwasaki K, Endo M, Ohnishi Y, Okumura T, Kashiwa K, Kinoshita O, Kubota K, Seguchi O, Toda K, Nishioka H, Nishinaka T, Nishimura T, Hashimoto T, Hatano M, Higashi H, Higo T, Fujino T, Hori Y, Miyoshi T, Yamanaka M, Ohno T, Kimura T, Kyo S, Sakata Y, Nakatani T; JCS/JSCVS/JATS/JSVS Joint Working Group. JCS/JSCVS/JATS/JSVS 2021 Guideline on Implantable Left Ventricular Assist Device for Patients With Advanced Heart Failure. *Circ J* 2022; **86**: 1024-1058 [PMID: 35387921 DOI: 10.1253/circj.CJ-21-0880]

6 **Rosenbaum AN**, Agre KE, Pereira NL. Genetics of dilated cardiomyopathy: practical implications for heart failure management. *Nat Rev Cardiol* 2020; **17**: 286-297 [PMID: 31605094 DOI: 10.1038/s41569-019-0284-0]

7 **Marian AJ**, Braunwald E. Hypertrophic Cardiomyopathy: Genetics, Pathogenesis, Clinical Manifestations, Diagnosis, and Therapy. *Circ Res* 2017; **121**: 749-770 [PMID: 28912181 DOI: 10.1161/CIRCRESAHA.117.311059]

8 **McNally EM**, Mestroni L. Dilated Cardiomyopathy: Genetic Determinants and Mechanisms. *Circ Res* 2017; **121**: 731-748 [PMID: 28912180 DOI: 10.1161/CIRCRESAHA.116.309396]

9 **Corrado D**, Basso C, Judge DP. Arrhythmogenic Cardiomyopathy. *Circ Res* 2017; **121**: 784-802 [PMID: 28912183 DOI: 10.1161/CIRCRESAHA.117.309345]

10 **Watkins H**, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med* 2011; **364**: 1643-1656 [PMID: 21524215 DOI: 10.1056/NEJMra0902923]

11 **Austin KM**, Trembley MA, Chandler SF, Sanders SP, Saffitz JE, Abrams DJ, Pu WT. Molecular mechanisms of arrhythmogenic cardiomyopathy. *Nat Rev Cardiol* 2019; **16**: 519-537 [PMID: 31028357 DOI: 10.1038/s41569-019-0200-7]

12 **Kitaoka H**, Tsutsui H, Kubo T, Ide T, Chikamori T, Fukuda K, Fujino N, Higo T, Isobe M, Kamiya C, Kato S, Kihara Y, Kinugawa K, Kinugawa S, Kogaki S, Komuro I, Hagiwara N, Ono M, Maekawa Y, Makita S, Matsui Y, Matsushima S, Sakata Y, Sawa Y, Shimizu W, Teraoka K, Tsuchihashi-Makaya M, Ishibashi-Ueda H, Watanabe M, Yoshimura M, Fukusima A, Hida S, Hikoso S, Imamura T, Ishida H, Kawai M, Kitagawa T, Kohno T, Kurisu S, Nagata Y, Nakamura M, Morita H, Takano H, Shiga T, Takei Y, Yuasa S, Yamamoto T, Watanabe T, Akasaka T, Doi Y, Kimura T, Kitakaze M, Kosuge M, Takayama M, Tomoike H; Japanese Circulation Society Joint Working Group. JCS/JHFS 2018 Guideline on the Diagnosis and Treatment of Cardiomyopathies. *Circ J* 2021; **85**: 1590-1689 [PMID: 34305070 DOI:

10.1253/circj.CJ-20-0910]
13 **Khush KK**, Cherikh WS, Chambers DC, Harhay MO, Hayes D Jr, Hsich E, Meiser B, Potena L, Robinson A, Rossano JW, Sadavarte A, Singh TP, Zuckermann A, Stehlik J; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report - 2019; focus theme: Donor and recipient size match. *J Heart Lung Transplant* 2019; **38**: 1056-1066 [PMID: 31548031 DOI: 10.1016/j.healun.2019.08.004]
14 **Nakatani T**, Fukushima N, Ono M, Saiki Y, Matsuda H, Nunoda S, Sawa Y, Isobe M. The Registry Report of Heart Transplantation in Japan (1999-2014). *Circ J* 2016; **80**: 44-50 [PMID: 26638870 DOI: 10.1253/circj.CJ-15-0975]
15 **Giuliano K**, Scheel P 3rd, Etchill E, Fraser CD 3rd, Suarez-Pierre A, Hsu S, Wittstein IS, Kasper EK, Florido R, Tandri H, Calkins H, Choi CW, Sharma K, Kilic A, Gilotra NA. Heart transplantation outcomes in arrhythmogenic right ventricular cardiomyopathy: a contemporary national analysis. *ESC Heart Fail* 2022; **9**: 988-997 [PMID: 35132806 DOI: 10.1002/ehf2.13687]
16 **Coppini R**, Ho CY, Ashley E, Day S, Ferrantini C, Girolami F, Tomberli B, Bardi S, Torricelli F, Cecchi F, Mugelli A, Poggesi C, Tardiff J, Olivotto I. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J Am Coll Cardiol* 2014; **64**: 2589-2600 [PMID: 25524337 DOI: 10.1016/j.jacc.2014.09.059]
17 **Marstrand P**, Han L, Day SM, Olivotto I, Ashley EA, Michels M, Pereira AC, Wittekind SG, Helms A, Saberi S, Jacoby D, Ware JS, Colan SD, Semsarian C, Ingles J, Lakdawala NK, Ho CY; SHaRe Investigators. Hypertrophic Cardiomyopathy With Left Ventricular Systolic Dysfunction: Insights From the SHaRe Registry. *Circulation* 2020; **141**: 1371-1383 [PMID: 32228044 DOI: 10.1161/CIRCULATIONAHA.119.044366]
18 **Haas J**, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, Feng Z, Müller S, Kayvanpour E, Vogel B, Sedaghat-Hamedani F, Lim WK, Zhao X, Fradkin D, Köhler D, Fischer S, Franke J, Marquart S, Barb I, Li DT, Amr A, Ehlermann P, Mereles D, Weis T, Hassel S, Kremer A, King V, Wirsz E, Isnard R, Komajda M, Serio A, Grasso M, Syrris P, Wicks E, Plagnol V, Lopes L, Gadgaard T, Eiskjær H, Jørgensen M, Garcia-Giustiniani D, Ortiz-Genga M, Crespo-Leiro MG, Deprez RH, Christiaans I, van Rijsingen IA, Wilde AA, Waldenstrom A, Bolognesi M, Bellazzi R, Mörner S, Bermejo JL, Monserrat L, Villard E, Mogensen J, Pinto YM, Charron P, Elliott P, Arbustini E, Katus HA, Meder B. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur Heart J* 2015; **36**: 1123-135a [PMID: 25163546 DOI: 10.1093/eurheartj/ehu301]
19 **Suwa Y**, Higo S, Nakamoto K, Sera F, Kunimatsu S, Masumura Y, Kanzaki M, Mizote I, Mizuno H, Fujio Y, Hikoso S, Sakata Y. Old-Age Onset Progressive Cardiac Contractile Dysfunction in a Patient with Polycystic Kidney Disease Harboring a PKD1 Frameshift Mutation. *Int Heart J* 2019; **60**: 220-225 [PMID: 30464138 DOI: 10.1536/ihj.18-184]
20 **Tobita T**, Nomura S, Fujita T, Morita H, Asano Y, Onoue K, Ito M, Imai Y, Suzuki A, Ko T, Satoh M, Fujita K, Naito AT, Furutani Y, Toko H, Harada M, Amiya E, Hatano M, Takimoto E, Shiga T, Nakanishi T, Sakata Y, Ono M, Saito Y, Takashima S, Hagiwara N, Aburatani H, Komuro I. Genetic basis of cardiomyopathy and the genotypes involved in prognosis and left ventricular reverse remodeling. *Sci Rep* 2018; **8**: 1998 [PMID: 29386531 DOI: 10.1038/s41598-018-20114-9]
21 **Towbin JA**, McKenna WJ, Abrams DJ, Ackerman MJ, Calkins H, Darrieux FCC, Daubert JP, de Chillou C, DePasquale EC, Desai MY, Estes NAM 3rd, Hua W, Indik JH, Ingles J, James CA, John RM, Judge DP, Keegan R, Krahn AD, Link MS, Marcus FI, McLeod CJ, Mestroni L, Priori SG, Saffitz JE, Sanatani S, Shimizu W, van Tintelen JP, Wilde AAM, Zareba W. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm* 2019; **16**: e301-e372 [PMID: 31078652 DOI: 10.1016/j.hrthm.2019.05.007]
22 **Awad MM**, Calkins H, Judge DP. Mechanisms of disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 258-267 [PMID: 18382419 DOI: 10.1038/ncpcardio1182]
23 **Al-Jassar C**, Bikker H, Overduin M, Chidgey M. Mechanistic basis of desmosome-targeted diseases. *J Mol Biol* 2013; **425**: 4006-4022 [PMID: 23911551 DOI: 10.1016/j.jmb.2013.07.035]
24 **Delmar M**, McKenna WJ. The cardiac desmosome and arrhythmogenic cardiomyopathies: from gene to disease. *Circ Res* 2010; **107**: 700-714 [PMID: 20847325 DOI: 10.1161/CIRCRESAHA.110.223412]
25 **Pugh TJ**, Kelly MA, Gowrisankar S, Hynes E, Seidman MA, Baxter SM, Bowser M, Harrison B, Aaron D, Mahanta LM, Lakdawala NK, McDermott G, White ET, Rehm HL, Lebo M, Funke BH. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet Med* 2014; **16**: 601-608 [PMID: 24503780 DOI: 10.1038/gim.2013.204]
26 **Garcia-Pavia P**, Syrris P, Salas C, Evans A, Mirelis JG, Cobo-Marcos M, Vilches C, Bornstein B, Segovia J, Alonso-Pulpon L, Elliott PM. Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study. *Heart* 2011; **97**: 1744-1752 [PMID: 21859740 DOI: 10.1136/hrt.2011.227967]
27 **Rigato I**, Bauce B, Rampazzo A, Zorzi A, Pilichou K, Mazzotti E, Migliore F, Marra MP, Lorenzon A, De Bortoli M, Calore M, Nava A, Daliento L, Gregori D, Iliceto S, Thiene G, Basso C, Corrado D. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet* 2013; **6**: 533-542 [PMID: 24070718 DOI: 10.1161/CIRCGENETICS.113.000288]
28 **Gandjbakhch E**, Redheuil A, Pousset F, Charron P, Frank R. Clinical Diagnosis, Imaging, and Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2018; **72**: 784-804 [PMID: 30092956 DOI: 10.1016/j.jacc.2018.05.065]
29 **Chen K**, Rao M, Guo G, Duru F, Chen L, Chen X, Song J, Hu S. Recessive variants in plakophilin-2 contributes to early-onset arrhythmogenic cardiomyopathy with severe heart failure. *Europace* 2019; **21**: 970-977 [PMID: 30830208 DOI: 10.1093/europace/euz026]
30 **Shiba M**, Higo S, Kondo T, Li J, Liu L, Ikeda Y, Kohama Y, Kameda S, Tabata T, Inoue H, Nakamura S, Takeda M, Ito E, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Phenotypic recapitulation and correction of desmoglein-2-deficient cardiomyopathy using human-induced pluripotent stem cell-derived cardiomyocytes. *Hum Mol Genet* 2021; **30**: 1384-1397 [PMID: 33949662 DOI: 10.1093/hmg/ddab127]

31 **Eshkind L**, Tian Q, Schmidt A, Franke WW, Windoffer R, Leube RE. Loss of desmoglein 2 suggests essential functions for early embryonic development and proliferation of embryonal stem cells. *Eur J Cell Biol* 2002; **81**: 592-598 [PMID: 12494996 DOI: 10.1078/0171-9335-00278]

32 **Sayed N**, Liu C, Wu JC. Translation of Human-Induced Pluripotent Stem Cells: From Clinical Trial in a Dish to Precision Medicine. *J Am Coll Cardiol* 2016; **67**: 2161-2176 [PMID: 27151349 DOI: 10.1016/j.jacc.2016.01.083]

33 **Kondo T**, Higo S, Shiba M, Kohama Y, Kameda S, Tabata T, Inoue H, Okuno S, Ogawa S, Nakamura S, Takeda M, Ito E, Li J, Liu L, Kuramoto Y, Lee JK, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Human-Induced Pluripotent Stem Cell-Derived Cardiomyocyte Model for TNNT2 Δ160E-Induced Cardiomyopathy. *Circ Genom Precis Med* 2022; **15**: e003522 [PMID: 35861968 DOI: 10.1161/CIRCGEN.121.003522]

34 **Tabata T**, Masumura Y, Higo S, Kunimatsu S, Kameda S, Inoue H, Okuno S, Ogawa S, Takashima S, Watanabe M, Miyagawa S, Hikoso S, Sakata Y. Multiplexed measurement of cell type-specific calcium kinetics using high-content image analysis combined with targeted gene disruption. *Biochem Biophys Res Commun* 2022; **637**: 40-49 [PMID: 36375249 DOI: 10.1016/j.bbrc.2022.10.088]

35 **Higo S**, Hikoso S, Miyagawa S, Sakata Y. Genome Editing in Human Induced Pluripotent Stem Cells (hiPSCs). *Methods Mol Biol* 2021; **2320**: 235-245 [PMID: 34302662 DOI: 10.1007/978-1-0716-1484-6_21]

36 **Padrón-Barthe L**, Domínguez F, Garcia-Pavia P, Lara-Pezzi E. Animal models of arrhythmogenic right ventricular cardiomyopathy: what have we learned and where do we go? *Basic Res Cardiol* 2017; **112**: 50 [PMID: 28688053 DOI: 10.1007/s00395-017-0640-3]

37 **Ohno S**, Nagaoka I, Fukuyama M, Kimura H, Itoh H, Makiyama T, Shimizu A, Horie M. Age-dependent clinical and genetic characteristics in Japanese patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circ J* 2013; **77**: 1534-1542 [PMID: 23514727 DOI: 10.1253/circj.cj-12-1446]

38 **Groeneweg JA**, Bhonsale A, James CA, te Riele AS, Dooijes D, Tichnell C, Murray B, Wiesfeld AC, Sawant AC, Kassamali B, Atsma DE, Volders PG, de Groot NM, de Boer K, Zimmerman SL, Kamel IR, van der Heijden JF, Russell SD, Jan Cramer M, Tedford RJ, Doevendans PA, van Veen TA, Tandri H, Wilde AA, Judge DP, van Tintelen JP, Hauer RN, Calkins H. Clinical Presentation, Long-Term Follow-Up, and Outcomes of 1001 Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Patients and Family Members. *Circ Cardiovasc Genet* 2015; **8**: 437-446 [PMID: 25820315 DOI: 10.1161/CIRCGENETICS.114.001003]

39 **Novelli V**, Malkani K, Cerrone M. Pleiotropic Phenotypes Associated With PKP2 Variants. *Front Cardiovasc Med* 2018; **5**: 184 [PMID: 30619891 DOI: 10.3389/fcvm.2018.00184]

40 **El-Battrawy I**, Zhao Z, Lan H, Cyganek L, Tombers C, Li X, Buljubasic F, Lang S, Tiburcy M, Zimmermann WH, Utikal J, Wieland T, Borggrefe M, Zhou XB, Akin I. Electrical dysfunctions in human-induced pluripotent stem cell-derived cardiomyocytes from a patient with an arrhythmogenic right ventricular cardiomyopathy. *Europace* 2018; **20**: f46-f56 [PMID: 29566126 DOI: 10.1093/europace/euy042]

41 **Buljubasic F**, El-Battrawy I, Lan H, Lomada SK, Chatterjee A, Zhao Z, Li X, Zhong R, Xu Q, Huang M, Liao Z, Lang S, Cyganek L, Zhou X, Wieland T, Borggrefe M, Akin I. Nucleoside Diphosphate Kinase B Contributes to Arrhythmogenesis in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes from a Patient with Arrhythmogenic Right Ventricular Cardiomyopathy. *J Clin Med* 2020; **9** [PMID: 32050722 DOI: 10.3390/jcm9020486]

42 **Ng R**, Manring H, Papoutsidakis N, Albertelli T, Tsai N, See CJ, Li X, Park J, Stevens TL, Bobbili PJ, Riaz M, Ren Y, Stoddard CE, Janssen PM, Bunch TJ, Hall SP, Lo YC, Jacoby DL, Qyang Y, Wright N, Ackermann MA, Campbell SG. Patient mutations linked to arrhythmogenic cardiomyopathy enhance calpain-mediated desmoplakin degradation. *JCI Insight* 2019; **5** [PMID: 31194698 DOI: 10.1172/jci.insight.128643]

43 **Xia S**, Wang X, Yue P, Li Y, Zhang D. Establishment of induced pluripotent stem cell lines from a family of an ARVC patient receiving heart transplantation in infant age carrying compound heterozygous mutations in DSP gene. *Stem Cell Res* 2020; **48**: 101977 [PMID: 32942234 DOI: 10.1016/j.scr.2020.101977]

44 **Moreau A**, Reisqs JB, Delanoe-Ayari H, Pierre M, Janin A, Deliniere A, Bessière F, Meli AC, Charrabi A, Lafont E, Valla C, Bauer D, Morel E, Gache V, Millat G, Nissan X, Faucherre A, Jopling C, Richard S, Mejat A, Chevalier P. Deciphering DSC2 arrhythmogenic cardiomyopathy electrical instability: From ion channels to ECG and tailored drug therapy. *Clin Transl Med* 2021; **11**: e319 [PMID: 33784018 DOI: 10.1002/ctm2.319]

45 **Reisqs JB**, Moreau A, Charrabi A, Sleiman Y, Meli AC, Millat G, Briand V, Beauverger P, Richard S, Chevalier P. The PPARγ pathway determines electrophysiological remodelling and arrhythmia risks in DSC2 arrhythmogenic cardiomyopathy. *Clin Transl Med* 2022; **12**: e748 [PMID: 35297182 DOI: 10.1002/ctm2.748]

46 **Caspi O**, Huber I, Gepstein A, Arbel G, Maizels L, Boulos M, Gepstein L. Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. *Circ Cardiovasc Genet* 2013; **6**: 557-568 [PMID: 24200905 DOI: 10.1161/CIRCGENETICS.113.000188]

47 **Kim C**, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 2013; **494**: 105-110 [PMID: 23354045 DOI: 10.1038/nature11799]

48 **Ma D**, Wei H, Lu J, Ho S, Zhang G, Sun X, Oh Y, Tan SH, Ng ML, Shim W, Wong P, Liew R. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2013; **34**: 1122-1133 [PMID: 22798562 DOI: 10.1093/eurheartj/ehs226]

49 **Dorn T**, Kornherr J, Parrotta EI, Zawada D, Ayetey H, Santamaria G, Iop L, Mastantuono E, Sinnecker D, Goedel A, Dirschinger RJ, My I, Laue S, Bozoglu T, Baarlink C, Ziegler T, Graf E, Hinkel R, Cuda G, Kääb S, Grace AA, Grosse R, Kupatt C, Meitinger T, Smith AG, Laugwitz KL, Moretti A. Interplay of cell-cell contacts and RhoA/MRTF-A signaling regulates cardiomyocyte identity. *EMBO J* 2018; **37** [PMID: 29764980 DOI: 10.15252/embj.201798133]

50 **Blazeski A**, Lowenthal J, Wang Y, Teuben R, Zhu R, Gerecht S, Tomaselli G, Tung L. Engineered Heart Slice Model of Arrhythmogenic Cardiomyopathy Using Plakophilin-2 Mutant Myocytes. *Tissue Eng Part A* 2019; **25**: 725-735 [PMID: 30520705 DOI: 10.1089/ten.TEA.2018.0272]

51 **Martewicz S**, Luni C, Serena E, Pavan P, Chen HV, Rampazzo A, Elvassore N. Transcriptomic Characterization of a

Human In Vitro Model of Arrhythmogenic Cardiomyopathy Under Topological and Mechanical Stimuli. *Ann Biomed Eng* 2019; **47**: 852-865 [PMID: 30569242 DOI: 10.1007/s10439-018-02134-8]

52 **Khudiakov A**, Zaytseva A, Perepelina K, Smolina N, Pervunina T, Vasichkina E, Karpushev A, Tomilin A, Malashicheva A, Kostareva A. Sodium current abnormalities and deregulation of Wnt/β-catenin signaling in iPSC-derived cardiomyocytes generated from patient with arrhythmogenic cardiomyopathy harboring compound genetic variants in plakophilin 2 gene. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165915 [PMID: 32768677 DOI: 10.1016/j.bbadis.2020.165915]

53 **Zhang K**, Cloonan PE, Sundaram S, Liu F, Das SL, Ewoldt JK, Bays JL, Tomp S, Toepfer CN, Marsiglia JDC, Gorham J, Reichart D, Eyckmans J, Seidman JG, Seidman CE, Chen CS. Plakophilin-2 truncating variants impair cardiac contractility by disrupting sarcomere stability and organization. *Sci Adv* 2021; **7**: eabh3995 [PMID: 34652945 DOI: 10.1126/sciadv.abh3995]

54 **Inoue H**, Nakamura S, Higo S, Shiba M, Kohama Y, Kondo T, Kameda S, Tabata T, Okuno S, Ikeda Y, Li J, Liu L, Yamazaki S, Takeda M, Ito E, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Modeling reduced contractility and impaired desmosome assembly due to plakophilin-2 deficiency using isogenic iPS cell-derived cardiomyocytes. *Stem Cell Reports* 2022; **17**: 337-351 [PMID: 35063130 DOI: 10.1016/j.stemcr.2021.12.016]

55 **Kirchner F**, Schuetz A, Boldt LH, Martens K, Dittmar G, Haverkamp W, Thierfelder L, Heinemann U, Gerull B. Molecular insights into arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 missense mutations. *Circ Cardiovasc Genet* 2012; **5**: 400-411 [PMID: 22781308 DOI: 10.1161/CIRCGENETICS.111.961854]

56 **Rasmussen TB**, Nissen PH, Palmfeldt J, Gehmlich K, Dalager S, Jensen UB, Kim WY, Heickendorff L, Mølgaard H, Jensen HK, Baandrup UT, Bross P, Mogensen J. Truncating plakophilin-2 mutations in arrhythmogenic cardiomyopathy are associated with protein haploinsufficiency in both myocardium and epidermis. *Circ Cardiovasc Genet* 2014; **7**: 230-240 [PMID: 24704780 DOI: 10.1161/CIRCGENETICS.113.000338]

57 **Corrado D**, Perazzolo Marra M, Zorzi A, Beffagna G, Cipriani A, Lazzari M, Migliore F, Pilichou K, Rampazzo A, Rigato I, Rizzo S, Thiene G, Anastasakis A, Asimaki A, Bucciarelli-Ducci C, Haugaa KH, Marchlinski FE, Mazzanti A, McKenna WJ, Pantazis A, Pelliccia A, Schmied C, Sharma S, Wichter T, Bauce B, Basso C. Diagnosis of arrhythmogenic cardiomyopathy: The Padua criteria. *Int J Cardiol* 2020; **319**: 106-114 [PMID: 32561223 DOI: 10.1016/j.ijcard.2020.06.005]

58 **Burridge PW**, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, Diecke S, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC. Chemically defined generation of human cardiomyocytes. *Nat Methods* 2014; **11**: 855-860 [PMID: 24930130 DOI: 10.1038/nmeth.2999]

59 **Gintant G**, Burridge P, Gepstein L, Harding S, Herron T, Hong C, Jalife J, Wu JC. Use of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in Preclinical Cancer Drug Cardiotoxicity Testing: A Scientific Statement From the American Heart Association. *Circ Res* 2019; **125**: e75-e92 [PMID: 31533542 DOI: 10.1161/RES.0000000000000291]

60 **Pérez-Hernández M**, van Opbergen CJM, Bagwan N, Vissing CR, Marrón-Liñares GM, Zhang M, Torres Vega E, Sorrentino A, Drici L, Sulek K, Zhai R, Hansen FB, Christensen AH, Boesgaard S, Gustafsson F, Rossing K, Small EM, Davies MJ, Rothenberg E, Sato PY, Cerrone M, Jensen THL, Qvortrup K, Bundgaard H, Delmar M, Lundby A. Loss of Nuclear Envelope Integrity and Increased Oxidant Production Cause DNA Damage in Adult Hearts Deficient in PKP2: A Molecular Substrate of ARVC. *Circulation* 2022; **146**: 851-867 [PMID: 35959657 DOI: 10.1161/CIRCULATIONAHA.122.060454]

61 **Stroud MJ**. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. *Biophys Rev* 2018; **10**: 1033-1051 [PMID: 29869195 DOI: 10.1007/s12551-018-0431-6]

62 **Ishizu T**, Higo S, Masumura Y, Kohama Y, Shiba M, Higo T, Shibamoto M, Nakagawa A, Morimoto S, Takashima S, Hikoso S, Sakata Y. Targeted Genome Replacement *via* Homology-directed Repair in Non-dividing Cardiomyocytes. *Sci Rep* 2017; **7**: 9363 [PMID: 28839205 DOI: 10.1038/s41598-017-09716-x]

63 **Kohama Y**, Higo S, Masumura Y, Shiba M, Kondo T, Ishizu T, Higo T, Nakamura S, Kameda S, Tabata T, Inoue H, Motooka D, Okuzaki D, Takashima S, Miyagawa S, Sawa Y, Hikoso S, Sakata Y. Adeno-associated virus-mediated gene delivery promotes S-phase entry-independent precise targeted integration in cardiomyocytes. *Sci Rep* 2020; **10**: 15348 [PMID: 32948788 DOI: 10.1038/s41598-020-72216-y]

64 **Tilemann L**, Ishikawa K, Weber T, Hajjar RJ. Gene therapy for heart failure. *Circ Res* 2012; **110**: 777-793 [PMID: 22383712 DOI: 10.1161/CIRCRESAHA.111.252981]

65 **Ishikawa K**, Weber T, Hajjar RJ. Human Cardiac Gene Therapy. *Circ Res* 2018; **123**: 601-613 [PMID: 30355138 DOI: 10.1161/CIRCRESAHA.118.311587]

66 **Chamberlain K**, Riyad JM, Weber T. Cardiac gene therapy with adeno-associated virus-based vectors. *Curr Opin Cardiol* 2017; **32**: 275-282 [PMID: 28169951 DOI: 10.1097/HCO.0000000000000386]

67 **Greenberg B**, Yaroshinsky A, Zsebo KM, Butler J, Felker GM, Voors AA, Rudy JJ, Wagner K, Hajjar RJ. Design of a phase 2b trial of intracoronary administration of AAV1/SERCA2a in patients with advanced heart failure: the CUPID 2 trial (calcium up-regulation by percutaneous administration of gene therapy in cardiac disease phase 2b). *JACC Heart Fail* 2014; **2**: 84-92 [PMID: 24622121 DOI: 10.1016/j.jchf.2013.09.008]

68 **Hasenfuss G**, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* 2002; **34**: 951-969 [PMID: 12234765 DOI: 10.1006/jmcc.2002.2037]

69 **Kho C**, Lee A, Hajjar RJ. Altered sarcoplasmic reticulum calcium cycling--targets for heart failure therapy. *Nat Rev Cardiol* 2012; **9**: 717-733 [PMID: 23090087 DOI: 10.1038/nrcardio.2012.145]

70 **Eisner D**, Caldwell J, Trafford A. Sarcoplasmic reticulum Ca-ATPase and heart failure 20 years later. *Circ Res* 2013; **113**: 958-961 [PMID: 24071456 DOI: 10.1161/CIRCRESAHA.113.302187]

71 **del Monte F**, Williams E, Lebeche D, Schmidt U, Rosenzweig A, Gwathmey JK, Lewandowski ED, Hajjar RJ. Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase in a rat model of heart failure. *Circulation* 2001; **104**: 1424-1429 [PMID: 11560860 DOI: 10.1161/hc3601.095574]

72 **Jessup M**, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, Hajjar RJ; Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Investigators.

Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. *Circulation* 2011; **124**: 304-313 [PMID: 21709064 DOI: 10.1161/CIRCULATIONAHA.111.022889]

73 **Cutler MJ**, Wan X, Plummer BN, Liu H, Deschenes I, Laurita KR, Hajjar RJ, Rosenbaum DS. Targeted sarcoplasmic reticulum Ca2+ ATPase 2a gene delivery to restore electrical stability in the failing heart. *Circulation* 2012; **126**: 2095-2104 [PMID: 23019291 DOI: 10.1161/CIRCULATIONAHA.111.071480]

74 **Greenberg B**, Butler J, Felker GM, Ponikowski P, Voors AA, Desai AS, Barnard D, Bouchard A, Jaski B, Lyon AR, Pogoda JM, Rudy JJ, Zsebo KM. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet* 2016; **387**: 1178-1186 [PMID: 26803443 DOI: 10.1016/S0140-6736(16)00082-9]

75 **Hulot JS**, Salem JE, Redheuil A, Collet JP, Varnous S, Jourdain P, Logeart D, Gandjbakhch E, Bernard C, Hatem SN, Isnard R, Cluzel P, Le Feuvre C, Leprince P, Hammoudi N, Lemoine FM, Klatzmann D, Vicaut E, Komajda M, Montalescot G, Lompré AM, Hajjar RJ; AGENT-HF Investigators. Effect of intracoronary administration of AAV1/SERCA2a on ventricular remodelling in patients with advanced systolic heart failure: results from the AGENT-HF randomized phase 2 trial. *Eur J Heart Fail* 2017; **19**: 1534-1541 [PMID: 28393439 DOI: 10.1002/ejhf.826]

76 **Lyon AR**, Babalis D, Morley-Smith AC, Hedger M, Suarez Barrientos A, Foldes G, Couch LS, Chowdhury RA, Tzortzis KN, Peters NS, Rog-Zielinska EA, Yang HY, Welch S, Bowles CT, Rahman Haley S, Bell AR, Rice A, Sasikaran T, Johnson NA, Falaschetti E, Parameshwar J, Lewis C, Tsui S, Simon A, Pepper J, Rudy JJ, Zsebo KM, Macleod KT, Terracciano CM, Hajjar RJ, Banner N, Harding SE. Investigation of the safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic heart failure supported with a left ventricular assist device - the SERCA-LVAD TRIAL. *Gene Ther* 2020; **27**: 579-590 [PMID: 32669717 DOI: 10.1038/s41434-020-0171-7]

77 **Repetti GG**, Toepfer CN, Seidman JG, Seidman CE. Novel Therapies for Prevention and Early Treatment of Cardiomyopathies. *Circ Res* 2019; **124**: 1536-1550 [PMID: 31120825 DOI: 10.1161/CIRCRESAHA.119.313569]

78 **Hakui H**, Kioka H, Miyashita Y, Nishimura S, Matsuoka K, Kato H, Tsukamoto O, Kuramoto Y, Takuwa A, Takahashi Y, Saito S, Ohta K, Asanuma H, Fu HY, Shinomiya H, Yamada N, Ohtani T, Sawa Y, Kitakaze M, Takashima S, Sakata Y, Asano Y. Loss-of-function mutations in the co-chaperone protein BAG5 cause dilated cardiomyopathy requiring heart transplantation. *Sci Transl Med* 2022; **14**: eabf3274 [PMID: 35044787 DOI: 10.1126/scitranslmed.abf3274]

79 **Calkins H**, Corrado D, Marcus F. Risk Stratification in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation* 2017; **136**: 2068-2082 [PMID: 29158215 DOI: 10.1161/CIRCULATIONAHA.117.030792]

80 **van Tintelen JP**, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, van der Smagt J, Boven LG, Mannens MM, van Langen IM, Hofstra RM, Otterspoor LC, Doevendans PA, Rodriguez LM, van Gelder IC, Hauer RN. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2006; **113**: 1650-1658 [PMID: 16567567 DOI: 10.1161/CIRCULATIONAHA.105.609719]

81 **Ramond F**, Janin A, Di Filippo S, Chanavat V, Chalabreysse L, Roux-Buisson N, Sanlaville D, Touraine R, Millat G. Homozygous PKP2 deletion associated with neonatal left ventricle noncompaction. *Clin Genet* 2017; **91**: 126-130 [PMID: 27030002 DOI: 10.1111/cge.12780]

82 **Katanyuwong P**, Khongkraparn A, Wattanasirichaigoon D. A Novel Homozygous PKP2 Variant in Severe Neonatal Non-compaction and Concomitant Ventricular Septal Defect: A Case Report. *Front Pediatr* 2021; **9**: 801491 [PMID: 35059364 DOI: 10.3389/fped.2021.801491]

83 **Verhagen JMA**, van den Born M, Kurul S, Asimaki A, van de Laar IMBH, Frohn-Mulder IME, Kammeraad JAE, Yap SC, Bartelings MM, van Slegtenhorst MA, von der Thüsen JH, Wessels MW. Homozygous Truncating Variant in PKP2 Causes Hypoplastic Left Heart Syndrome. *Circ Genom Precis Med* 2018; **11**: e002397 [PMID: 30562116 DOI: 10.1161/CIRCGEN.118.002397]

84 **Li J**, Zhang L, Yu L, Minami I, Miyagawa S, Hörning M, Dong J, Qiao J, Qu X, Hua Y, Fujimoto N, Shiba Y, Zhao Y, Tang F, Chen Y, Sawa Y, Tang C, Liu L. Circulating re-entrant waves promote maturation of hiPSC-derived cardiomyocytes in self-organized tissue ring. *Commun Biol* 2020; **3**: 122 [PMID: 32170165 DOI: 10.1038/s42003-020-0853-0]

85 **Li J**, Minami I, Shiozaki M, Yu L, Yajima S, Miyagawa S, Shiba Y, Morone N, Fukushima S, Yoshioka M, Li S, Qiao J, Li X, Wang L, Kotera H, Nakatsuji N, Sawa Y, Chen Y, Liu L. Human Pluripotent Stem Cell-Derived Cardiac Tissue-like Constructs for Repairing the Infarcted Myocardium. *Stem Cell Reports* 2017; **9**: 1546-1559 [PMID: 29107590 DOI: 10.1016/j.stemcr.2017.09.007]

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MINIREVIEWS

Mesenchymal stem/stromal cells-derived exosomes for osteoporosis treatment

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Abstract

Osteoporosis is a systemic bone disease, which leads to decreased bone mass and an increased risk of fragility fractures. Currently, there are many anti-resorption drugs and osteosynthesis drugs, which are effective in the treatment of osteoporosis, but their usage is limited due to their contraindications and side effects. In regenerative medicine, the unique repair ability of mesenchymal stem cells (MSCs) has been favored by researchers. The exosomes secreted by MSCs have signal transduction and molecular delivery mechanisms, which may have therapeutic effects. In this review, we describe the regulatory effects of MSCs-derived exosomes on osteoclasts, osteoblasts, and bone immunity. We aim to summarize the preclinical studies of exosome therapy in osteoporosis. Furthermore, we speculate that exosome therapy can be a future direction to improve bone health.

Key Words: Mesenchymal stem cells; Exosome; Osteoporosis; Osteoblasts; Osteoclasts; Bone immunity

Core Tip: Osteoporosis is one of the major diseases endangering bone health in the elderly. The existing treatment drugs have problems such as long-term administration and side effects; thus, it is fundamentally difficult to cure osteoporosis. Exosomes derived from mesenchymal stem cells (MSCs) are vesicles that deliver signals and molecules between cells and have shown substantial positive effects in pre-clinical trials. In this review, we summarize the latest progress of MSCs-derived exosomes in the regulation of bone metabolism.

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INTRODUCTION

Osteoporosis (OP) is a chronic systemic bone disease. It is characterized by decreased bone mass and destruction of bone microstructures, resulting in decreased bone mineral density (BMD) and ultimately an increased risk of fragility fractures[1]. The dynamic balance between bone formation and bone resorption is an important way to maintain normal bone metabolism. Osteoblasts promote bone formation by mineralizing the matrix, while osteoclasts degrade the bone matrix by secreting H+ and releasing cathepsin K, accelerating bone dissolution[2].

At present, the prevention and treatment of OP can be divided into adjuvant therapy and drug therapy. The therapeutic effects of drugs are mainly divided into two aspects: Inhibition of osteoclasts (inhibition of bone resorption) and stimulation of osteoblasts (promotion of bone formation). Among them, the drugs that inhibit bone resorption are denosumab, bisphosphonates and selective estrogen receptor modulators; and drugs that promote bone formation are teriparatide and abaloparatide. Romosozumab, a monoclonal antibody directed against sclerostin, has a dual regulatory effect, inhibiting bone resorption and promoting bone formation at the same time[3].

Although a variety of therapeutic drugs for OP have emerged, all the drugs mentioned above have side effects[4], and more novel and effective drugs and therapies are needed. There are new advances in the research on stem cells and their exosomes in tissue repair and treatment. Therefore, the new scheme of MSCs-derived exosomes for the treatment of OP has gradually become a therapeutic option[5].

EXOSOMES AND MESENCHYMAL STEM CELLS

Exosomes are small vesicles secreted by cells with a diameter of 40 to 160 nm, which carry signals and molecules among cells. In addition, the exosomes secreted by the cells in diseased states contain specific microRNAs (miRNAs), which is helpful for the diagnosis of diseases[6]. As a carrier of signal transduction and drug delivery, exosomes also have good potential in the treatment of diseases, and their effectiveness has been confirmed by numerous animal studies[7-13] (Figure 1). In 1974, Friedenstein *et al*[14] first discovered that mesenchymal stem cells (MSCs) in bone marrow have the potential for osteogenic differentiation. Later, researchers continued to explore the function of MSCs and found that MSCs are pluripotent stem cells, which have the ability to differentiate into osteoblasts, adipocytes, chondrocytes, cardiac muscle cells and skeletal muscle cells[15]. Previous studies have found that therapy with MSCs can accelerate bone tissue repair and regeneration and maintain bone mass in OP[16,17].

MSCs-derived exosomes are likely to play a major role in mediating the therapeutic effect of MSCs [18,19]. A schematic diagram illustrating the mechanisms of MSCs-derived exosomes in the treatment of OP is shown in Figure 2. Therefore, MSCs-derived exosomes are expected to be a new biological agent in the future.

MSCS-DERIVED EXOSOMES IN THE TREATMENT OF OP

Exosomes have the characteristics of good targeting ability, high permeability and low toxicity. Therefore, exosomes therapy has become one of the hot spots in the study of OP treatment. Bone regeneration is one of the main modalities in the treatment of OP and bone fracture. MSCs-derived exosomes can promote the formation of new bone with vasculature, biomechanics, and histology[20]. MSCs-derived exosomes regulate bone metabolism and treat OP through signaling pathways, such as stimulating osteoblast differentiation from bone marrow stem cells, promoting osteoblast proliferation,

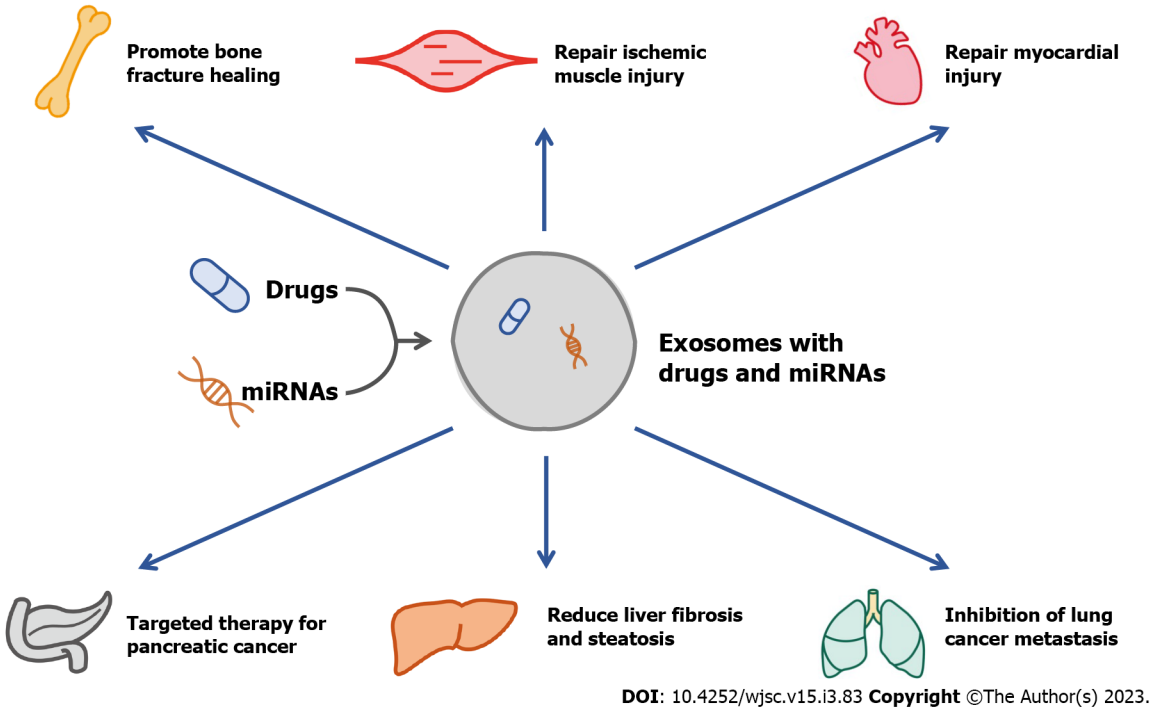

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Figure 1 Schematic diagram illustrating the therapeutic potential of exosomes in diseases of multiple organs. miRNAs: MicroRNAs.

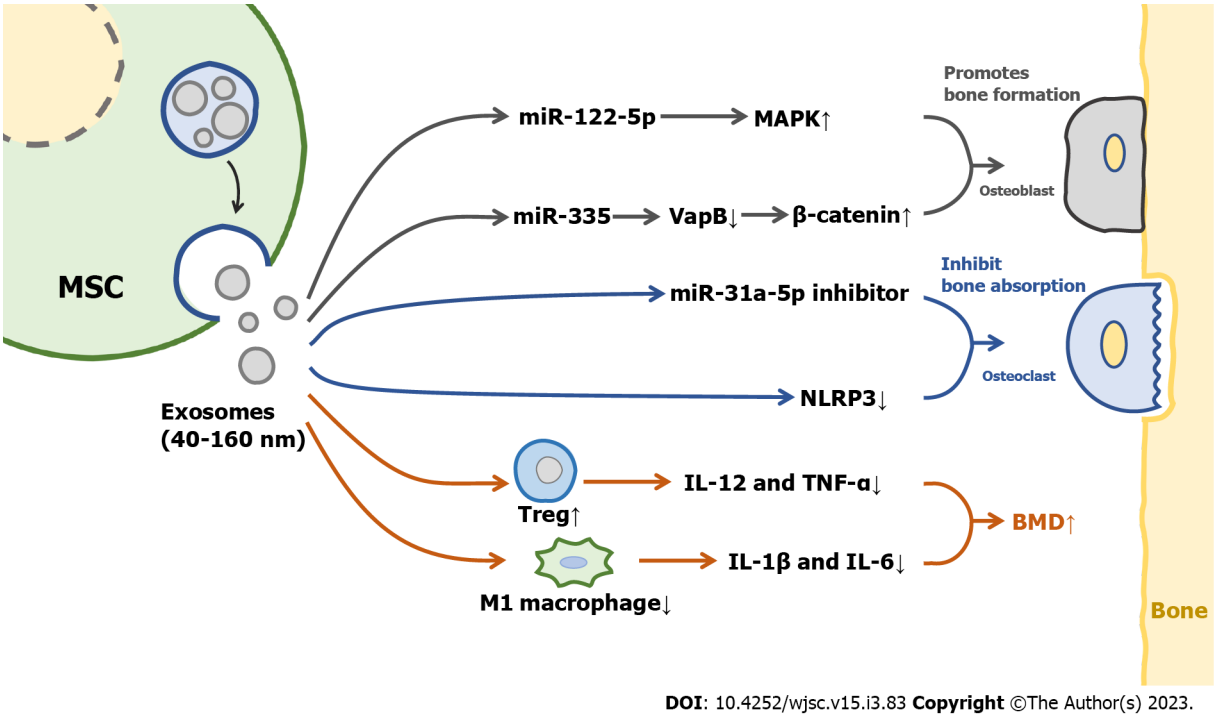

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Figure 2 Schematic diagram illustrating the mechanisms of mesenchymal stem cells-derived exosomes in the treatment of osteoporosis. MSC: Mesenchymal stem cell; BMD: Bone mineral density.

inducing angiogenesis, and immunomodulation[21]. During treatment, MSCs-derived exosomes achieve the purpose of bone regeneration and treatment of OP by carrying and transporting proteins, miRNAs, and artificial synthetic drugs[22-24]. According to the pathogenesis of OP, exosomes are mainly involved in regulating the effects of osteoblasts, osteoclasts and bone immunity (Table 1).

Table 1 Summary of studies on mesenchymal stem cells-derived exosomes in osteoporosis treatment

Ref.	Exosome source	Cell type	Consequence
Zuo *et al*[24], 2019	BMSCs	BMSCs (after irradiation)	Alleviating radiation-induced bone loss by restoring Wnt/β-Catenin signaling pathway
Zhao *et al*[25], 2018	BMSCs	Osteoblasts (hFOB 1.19)	Promoting proliferation of osteoblasts *via* MAPK pathway
Liao *et al*[26], 2019	BMSCs	Osteoblasts	Promoting proliferation of osteoblasts by carrying miR-122-5p
Qi *et al*[27], 2016	hiPSC-MSCs	Osteoblasts	Promoting osteoblast proliferation, differentiation and bone formation
Hu *et al*[28], 2021	BMSCs	Osteoblasts	Promoting differentiation of osteoblasts by carrying miR-335
Zhang *et al*[31], 2021	AD-MSCs	Osteoclasts	Inhibiting NLRP3 inflammasome activation in osteoclasts
Nakao *et al*[32], 2021	GMSCs	Osteoclasts	Inhibiting RANKL and reducing osteoclast formation by carrying miR-1260b *via* Wnt5a/JNK signal pathway
Xu *et al*[33], 2018	BMSCs	Osteoclasts	Increasing the number of osteoclasts by carrying miR-31a-5p *via* RhoA pathway
Wei *et al*[40], 2019	BMSCs	Osteoblasts	Promoting osteoblast differentiation by inhibiting macrophage polarization and reducing the levels of inflammatory factors

BMSCs: Bone marrow mesenchymal stem cells; hiPSC-MSCs: Mesenchymal stem cells derived from human induced pluripotent stem cells; AD-MSCs: Adipose derived mesenchymal stem cells; GMSCs: Gingival tissue-derived mesenchymal stem cells; MAPK: Mitogen-activated protein kinase; NLRP3: NLR family pyrin domain containing 3; RANKL: Receptor activator of NF-κB ligand; Wnt5a: Wnt family member 5A; JNK: c-Jun N-terminal kinases.

EFFECTS OF MSCS-DERIVED EXOSOMES ON OSTEOBLASTS

Osteoblasts are derived from pluripotent MSCs, which are major functional cells in bone matrix synthesis, secretion, and mineralization. Exosomes can directly regulate the activity of osteoblasts and then affect OP. MSCs-derived exosomes promoted the cell cycle of hFOB 1.19 cells, a type of osteoblast, and activated their proliferative activity through the MAPK pathway[25]. It was also found that overexpression of miR-122-5p in MSCs-derived exosomes of bone marrow can increase the BMD of the femoral head through the MAPK pathway[26]. Co-culture of MSCs-derived exosomes with bone marrow MSCs from osteoporotic rats resulted in increased levels of osteogenesis-related proteins and mineral deposition; in addition, the use of MSCs-derived exosomes promoted bone regeneration in a rat model of calvarial defects[27]. One study showed that the infusion of bone marrow MSCs-derived exosomes carrying miR-335 into mice with fractures significantly accelerated fracture healing, as miR-335 in exosomes was able to inhibit VapB, activate the Wnt/β-catenin pathway, and promote osteoblast differentiation[28]. Notably, exosomal miRNAs derived from MSCs are critical in regulating the function of osteoblasts. These miRNAs participate in and regulate some key signaling pathways and alter protein expression in osteoblasts. The positive roles of exosomes in osteoblast proliferation and differentiation, and the excellent therapeutic performance of exosomes in bone repair and bone mass recovery, indicate the potential of MSCs-derived exosomes in the treatment of OP.

EFFECTS OF MSCS-DERIVED EXOSOMES ON OSTEOCLASTS

Osteoclasts originate from hematopoietic progenitor cells in the bone marrow, and play an osteolytic role by secreting H+ and cathepsin K[29,30]. Adipose MSCs-derived exosomes inhibited NLRP3 in the osteoclasts of diabetic OP rat models and increased BMD[31]. RANKL is a major factor in promoting the differentiation of preosteoclasts into osteoclasts, gingiva MSCs-derived exosomes can target Wnt5a in periodontal osteoclasts and inhibit the expression of RANKL, thus reducing the differentiation of osteoclasts[32]. Of course, as a carrier of miRNA, exosomes will also have an effect on osteoclasts by carrying miRNA. Bone marrow MSCs-derived exosomes delivered miR-31a-5p to osteoclasts, which increased osteoclast number and bone resorption. In addition, BMD significantly increased in a rat model treated with miR-31a-5p inhibitor[33]. These findings suggested that MSCs-derived exosomes can regulate osteoclast differentiation, increase bone density, and inhibit OP. Notably, bone marrow stromal cell-derived exosomes do not have any effect on osteoclasts at the surface of trabecular bone, and therefore do not play a role in preventing bone resorption[34]. There are few studies on the regulation of osteoclast differentiation by MSCs-derived exosomes. Therefore, we need to further explore the potential of MSCs-derived exosomes in the treatment of OP by regulating osteoclast differ-

entiation.

EFFECTS OF MSCS-DERIVED EXOSOMES ON BONE IMMUNITY

There is an intricate relationship between the immune system and the skeletal system. Activated immune cells release inflammatory mediators and cytokines that upset the balance of bone remodeling and activate osteoclasts, leading to bone loss and OP[35,36]. MSCs-derived exosomes can regulate the immune system[37]. After treatment with adipose MSCs-derived exosomes in mice with colitis, regulatory T cells returned to normal baseline level, and the levels of inflammatory factors such as interleukin (IL)-12 and tumor necrosis factor-α reduced[38]. Exosomes derived from adipose and bone marrow MSCs can significantly increase the level of type II collagen in articular cartilage, promote articular cartilage formation and accelerate the recovery of osteoarthritis in mice[39]. MSCs-derived exosomes promote the differentiation of bone marrow MSCs into osteoblasts by inhibiting the polarization of M1 macrophages and reducing the levels of inflammatory cytokines such as Il-1β and IL-6[40]. MSCs-derived exosomes can regulate immunity and inflammation in vivo. Unfortunately, little is known about their role in bone immunity. However, available findings suggest the potential of MSCs-derived exosomes in the treatment of OP by modulating bone immunity.

CONCLUSION

At present, drug treatment of OP still focuses on inhibiting bone resorption and promoting bone formation. Drug therapy has a good effect, but long-term use of drugs can cause serious side effects. In the field of bone regeneration, stem cell therapy has significant efficacy, and exosomes are one of the important carriers of cell information and factors. Therefore, MSCs-derived exosomes may be a promising biological agent in the treatment of OP. This review focuses on the mechanism of MSCs-derived exosomes in the treatment of OP, by delivering miRNA, regulating related targets, and regulating bone immunity. Therefore, the potential of MSCs-derived exosomes in the treatment of OP is anticipated, but further preclinical studies are needed to prove the safety and reliability of exosome therapy and provide strong evidence for the clinical conversion of exosome therapy.

FOOTNOTES

Author contributions: Shao J and Zhang WW were responsible for the concept design; Huo KL, Shao J and Zhang WW wrote the main manuscript text and prepared Figures 1 and 2; Yang TY provided technical support; all authors reviewed the manuscript.

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REFERENCES

1 **Compston JE**, McClung MR, Leslie WD. Osteoporosis. *Lancet* 2019; **393**: 364-376 [PMID: 30696576 DOI: 10.1016/S0140-6736(18)32112-3]
2 **Manolagas SC**. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev* 2000; **21**: 115-137 [PMID: 10782361 DOI: 10.1210/edrv.21.2.0395]
3 **McClung MR**, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, Langdahl BL, Reginster JY, Zanchetta JR, Wasserman SM, Katz L, Maddox J, Yang YC, Libanati C, Bone HG. Romosozumab in postmenopausal women with low bone mineral density. *N Engl J Med* 2014; **370**: 412-420 [PMID: 24382002 DOI: 10.1056/NEJMoa1305224]
4 **Tella SH**, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol* 2014; **142**: 155-170 [PMID: 24176761 DOI: 10.1016/j.jsbmb.2013.09.008]
5 **Xie X**, Xiong Y, Panayi AC, Hu L, Zhou W, Xue H, Lin Z, Chen L, Yan C, Mi B, Liu G. Exosomes as a Novel Approach to Reverse Osteoporosis: A Review of the Literature. *Front Bioeng Biotechnol* 2020; **8**: 594247 [PMID: 33195163 DOI: 10.3389/fbioe.2020.594247]
6 **Kong D**, Chen T, Zheng X, Yang T, Zhang Y, Shao J. Comparative profile of exosomal microRNAs in postmenopausal women with various bone mineral densities by small RNA sequencing. *Genomics* 2021; **113**: 1514-1521 [PMID: 33785399 DOI: 10.1016/j.ygeno.2021.03.028]
7 **Kalluri R**, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020; **367** [PMID: 32029601 DOI: 10.1126/science.aau6977]
8 **Liu W**, Li L, Rong Y, Qian D, Chen J, Zhou Z, Luo Y, Jiang D, Cheng L, Zhao S, Kong F, Wang J, Xu T, Gong F, Huang Y, Gu C, Zhao X, Bai J, Wang F, Zhao W, Zhang L, Li X, Yin G, Fan J, Cai W. Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. *Acta Biomater* 2020; **103**: 196-212 [PMID: 31857259 DOI: 10.1016/j.actbio.2019.12.020]
9 **Yan B**, Zhang Y, Liang C, Liu B, Ding F, Wang Y, Zhu B, Zhao R, Yu XY, Li Y. Stem cell-derived exosomes prevent pyroptosis and repair ischemic muscle injury through a novel exosome/circHIPK3/ FOXO3a pathway. *Theranostics* 2020; **10**: 6728-6742 [PMID: 32550900 DOI: 10.7150/thno.42259]
10 **Liu S**, Chen X, Bao L, Liu T, Yuan P, Yang X, Qiu X, Gooding JJ, Bai Y, Xiao J, Pu F, Jin Y. Treatment of infarcted heart tissue *via* the capture and local delivery of circulating exosomes through antibody-conjugated magnetic nanoparticles. *Nat Biomed Eng* 2020; **4**: 1063-1075 [PMID: 33159193 DOI: 10.1038/s41551-020-00637-1]
11 **Kamerkar S**, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, Lee JJ, Kalluri R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 2017; **546**: 498-503 [PMID: 28607485 DOI: 10.1038/nature22341]
12 **Gao H**, Jin Z, Bandyopadhyay G, Cunha E Rocha K, Liu X, Zhao H, Zhang D, Jouihan H, Pourshahian S, Kisseleva T, Brenner DA, Ying W, Olefsky JM. MiR-690 treatment causes decreased fibrosis and steatosis and restores specific Kupffer cell functions in NASH. *Cell Metab* 2022; **34**: 978-990.e4 [PMID: 35700738 DOI: 10.1016/j.cmet.2022.05.008]
13 **Tian W**, Yang X, Yang H, Lv M, Sun X, Zhou B. Exosomal miR-338-3p suppresses non-small-cell lung cancer cells metastasis by inhibiting CHL1 through the MAPK signaling pathway. *Cell Death Dis* 2021; **12**: 1030 [PMID: 34718336 DOI: 10.1038/s41419-021-04314-2]
14 **Friedenstein AJ**, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning *in vitro* and retransplantation in vivo. *Transplantation* 1974; **17**: 331-340 [PMID: 4150881 DOI: 10.1097/00007890-197404000-00001]
15 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736 [PMID: 19172693 DOI: 10.1038/nri2395]
16 **Chen T**, Yang T, Zhang W, Shao J. The therapeutic potential of mesenchymal stem cells in treating osteoporosis. *Biol Res* 2021; **54**: 42 [PMID: 34930472 DOI: 10.1186/s40659-021-00366-y]
17 **Xu GP**, Zhang XF, Sun L, Chen EM. Current and future uses of skeletal stem cells for bone regeneration. *World J Stem Cells* 2020; **12**: 339-350 [PMID: 32547682 DOI: 10.4252/wjsc.v12.i5.339]
18 **Pegtel DM**, Gould SJ. Exosomes. *Annu Rev Biochem* 2019; **88**: 487-514 [PMID: 31220978 DOI: 10.1146/annurev-biochem-013118-111902]
19 **Álvarez-Viejo M**. Mesenchymal stem cells from different sources and their derived exosomes: A pre-clinical perspective. *World J Stem Cells* 2020; **12**: 100-109 [PMID: 32184935 DOI: 10.4252/wjsc.v12.i2.100]
20 **Tan SHS**, Wong JRY, Sim SJY, Tjio CKE, Wong KL, Chew JRJ, Hui JHP, Toh WS. Mesenchymal stem cell exosomes in bone regenerative strategies-a systematic review of preclinical studies. *Mater Today Bio* 2020; **7**: 100067 [PMID: 32695985 DOI: 10.1016/j.mtbio.2020.100067]
21 **Rudiansyah M**, El-Sehrawy AA, Ahmad I, Terefe EM, Abdelbasset WK, Bokov DO, Salazar A, Rizaev JA, Muthanna FMS, Shalaby MN. Osteoporosis treatment by mesenchymal stromal/stem cells and their exosomes: Emphasis on signaling pathways and mechanisms. *Life Sci* 2022; **306**: 120717 [PMID: 35792178 DOI: 10.1016/j.lfs.2022.120717]
22 **Chen L**, Mou S, Li F, Zeng Y, Sun Y, Horch RE, Wei W, Wang Z, Sun J. Self-Assembled Human Adipose-Derived Stem Cell-Derived Extracellular Vesicle-Functionalized Biotin-Doped Polypyrrole Titanium with Long-Term Stability and Potential Osteoinductive Ability. *ACS Appl Mater Interfaces* 2019; **11**: 46183-46196 [PMID: 31718127 DOI: 10.1021/acsami.9b17015]
23 **Li QC**, Li C, Zhang W, Pi W, Han N. Potential Effects of Exosomes and their MicroRNA Carrier on Osteoporosis. *Curr Pharm Des* 2022; **28**: 899-909 [PMID: 35088659 DOI: 10.2174/1381612828666220128104206]
24 **Zuo R**, Liu M, Wang Y, Li J, Wang W, Wu J, Sun C, Li B, Wang Z, Lan W, Zhang C, Shi C, Zhou Y. BM-MSC-derived exosomes alleviate radiation-induced bone loss by restoring the function of recipient BM-MSCs and activating Wnt/β-catenin signaling. *Stem Cell Res Ther* 2019; **10**: 30 [PMID: 30646958 DOI: 10.1186/s13287-018-1121-9]
25 **Zhao P**, Xiao L, Peng J, Qian YQ, Huang CC. Exosomes derived from bone marrow mesenchymal stem cells improve osteoporosis through promoting osteoblast proliferation *via* MAPK pathway. *Eur Rev Med Pharmacol Sci* 2018; **22**: 3962-3970 [PMID: 29949171 DOI: 10.26355/eurrev_201806_15280]

26 **Liao W**, Ning Y, Xu HJ, Zou WZ, Hu J, Liu XZ, Yang Y, Li ZH. BMSC-derived exosomes carrying microRNA-122-5p promote proliferation of osteoblasts in osteonecrosis of the femoral head. *Clin Sci (Lond)* 2019; **133**: 1955-1975 [PMID: 31387936 DOI: 10.1042/CS20181064]

27 **Qi X**, Zhang J, Yuan H, Xu Z, Li Q, Niu X, Hu B, Wang Y, Li X. Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats. *Int J Biol Sci* 2016; **12**: 836-849 [PMID: 27313497 DOI: 10.7150/ijbs.14809]

28 **Hu H**, Wang D, Li L, Yin H, He G, Zhang Y. Role of microRNA-335 carried by bone marrow mesenchymal stem cells-derived extracellular vesicles in bone fracture recovery. *Cell Death Dis* 2021; **12**: 156 [PMID: 33542183 DOI: 10.1038/s41419-021-03430-3]

29 **Boyle WJ**, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003; **423**: 337-342 [PMID: 12748652 DOI: 10.1038/nature01658]

30 **Lotinun S**, Kiviranta R, Matsubara T, Alzate JA, Neff L, Lüth A, Koskivirta I, Kleuser B, Vacher J, Vuorio E, Horne WC, Baron R. Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation. *J Clin Invest* 2013; **123**: 666-681 [PMID: 23321671 DOI: 10.1172/JCI64840]

31 **Zhang L**, Wang Q, Su H, Cheng J. Exosomes from adipose derived mesenchymal stem cells alleviate diabetic osteoporosis in rats through suppressing NLRP3 inflammasome activation in osteoclasts. *J Biosci Bioeng* 2021; **131**: 671-678 [PMID: 33849774 DOI: 10.1016/j.jbiosc.2021.02.007]

32 **Nakao Y**, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kou X, Chen C, Liu D, Watanabe Y, Hayashi C, Yamato H, Yotsumoto K, Tanaka U, Taketomi T, Uchiumi T, Le AD, Shi S, Nishimura F. Exosomes from TNF-α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. *Acta Biomater* 2021; **122**: 306-324 [PMID: 33359765 DOI: 10.1016/j.actbio.2020.12.046]

33 **Xu R**, Shen X, Si Y, Fu Y, Zhu W, Xiao T, Fu Z, Zhang P, Cheng J, Jiang H. MicroRNA-31a-5p from aging BMSCs links bone formation and resorption in the aged bone marrow microenvironment. *Aging Cell* 2018; **17**: e12794 [PMID: 29896785 DOI: 10.1111/acel.12794]

34 **Luo ZW**, Li FX, Liu YW, Rao SS, Yin H, Huang J, Chen CY, Hu Y, Zhang Y, Tan YJ, Yuan LQ, Chen TH, Liu HM, Cao J, Liu ZZ, Wang ZX, Xie H. Aptamer-functionalized exosomes from bone marrow stromal cells target bone to promote bone regeneration. *Nanoscale* 2019; **11**: 20884-20892 [PMID: 31660556 DOI: 10.1039/c9nr02791b]

35 **Arron JR**, Choi Y. Bone *versus* immune system. *Nature* 2000; **408**: 535-536 [PMID: 11117729 DOI: 10.1038/35046196]

36 **Srivastava RK**, Dar HY, Mishra PK. Immunoporosis: Immunology of Osteoporosis-Role of T Cells. *Front Immunol* 2018; **9**: 657 [PMID: 29675022 DOI: 10.3389/fimmu.2018.00657]

37 **Ha DH**, Kim HK, Lee J, Kwon HH, Park GH, Yang SH, Jung JY, Choi H, Lee JH, Sung S, Yi YW, Cho BS. Mesenchymal Stem/Stromal Cell-Derived Exosomes for Immunomodulatory Therapeutics and Skin Regeneration. *Cells* 2020; **9** [PMID: 32392899 DOI: 10.3390/cells9051157]

38 **Heidari N**, Abbasi-Kenarsari H, Namaki S, Baghaei K, Zali MR, Ghaffari Khaligh S, Hashemi SM. Adipose-derived mesenchymal stem cell-secreted exosome alleviates dextran sulfate sodium-induced acute colitis by Treg cell induction and inflammatory cytokine reduction. *J Cell Physiol* 2021; **236**: 5906-5920 [PMID: 33728664 DOI: 10.1002/jcp.30275]

39 **Fazaeli H**, Kalhor N, Naserpour L, Davoodi F, Sheykhhasan M, Hosseini SKE, Rabiei M, Sheikholeslami A. A Comparative Study on the Effect of Exosomes Secreted by Mesenchymal Stem Cells Derived from Adipose and Bone Marrow Tissues in the Treatment of Osteoarthritis-Induced Mouse Model. *Biomed Res Int* 2021; **2021**: 9688138 [PMID: 34616850 DOI: 10.1155/2021/9688138]

40 **Wei F**, Li Z, Crawford R, Xiao Y, Zhou Y. Immunoregulatory role of exosomes derived from differentiating mesenchymal stromal cells on inflammation and osteogenesis. *J Tissue Eng Regen Med* 2019; **13**: 1978-1991 [PMID: 31359542 DOI: 10.1002/term.2947]

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ORIGINAL ARTICLE

Basic Study

Mammalian Ste20-like kinase 1 inhibition as a cellular mediator of anoikis in mouse bone marrow mesenchymal stem cells

Tao Zhang, Qian Zhang, Wan-Cheng Yu

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Abstract

BACKGROUND

The low survival rate of mesenchymal stem cells (MSCs) caused by anoikis, a form of apoptosis, limits the therapeutic efficacy of MSCs. As a proapoptotic molecule, mammalian Ste20-like kinase 1 (Mst1) can increase the production of reactive oxygen species (ROS), thereby promoting anoikis. Recently, we found that Mst1 inhibition could protect mouse bone marrow MSCs (mBMSCs) from H_2O_2-induced cell apoptosis by inducing autophagy and reducing ROS production. However, the influence of Mst1 inhibition on anoikis in mBMSCs remains unclear.

AIM

To investigate the mechanisms by which Mst1 inhibition acts on anoikis in isolated mBMSCs.

METHODS

Poly-2-hydroxyethyl methacrylate-induced anoikis was used following the silencing of Mst1 expression by short hairpin RNA (shRNA) adenovirus transfection. Integrin (ITGs) were tested by flow cytometry. Autophagy and ITGα5β1 were inhibited using 3-methyladenine and small interfering RNA, respectively. The alterations in anoikis were measured by Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling and anoikis assays. The levels of the anoikis-related proteins ITGα5, ITGβ1, and phospho-focal adhesion kinase and the activation of caspase 3 and the autophagy-related proteins microtubules associated protein 1 light chain 3 II/I, Beclin1 and p62 were detected by Western blotting.

RESULTS

In isolated mBMSCs, Mst1 expression was upregulated, and Mst1 inhibition significantly reduced cell apoptosis, induced autophagy and decreased ROS levels. Mechanistically, we found that Mst1 inhibition could upregulate ITGα5

and ITGβ1 expression but not ITGα4, ITGαv, or ITGβ3 expression. Moreover, autophagy induced by upregulated ITGα5β1 expression following Mst1 inhibition played an essential role in the protective efficacy of Mst1 inhibition in averting anoikis.

CONCLUSION

Mst1 inhibition ameliorated autophagy formation, increased ITGα5β1 expression, and decreased the excessive production of ROS, thereby reducing cell apoptosis in isolated mBMSCs. Based on these results, Mst1 inhibition may provide a promising strategy to overcome anoikis of implanted MSCs.

Key Words: Mouse bone marrow mesenchymal stem cell; Mammalian sterile 20-like kinase 1; Anoikis; Integrin; Autophagy; Reactive oxygen species

Core Tip: In isolated mouse bone marrow mesenchymal stem cell (mBMSCs), Mammalian sterile 20-like kinase 1 (Mst1) inhibition could ameliorate not only autophagy formation but also upregulate integrin (ITG) α5β1 expression (but not ITGα4, ITGαv, or ITGβ3). In addition, Mst1 inhibition-induced autophagy could scavenge the excessive production of ITGα5β1-triggered ROS. Therefore, Mst1 inhibition-based infusion may improve the survival of MSCs, thereby serving as an ideal candidate for clinical transplantation in pulmonary arterial hypertension.

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INTRODUCTION

Mesenchymal stem cell (MSC) therapy is characterized by anti-inflammatory, immunomodulatory, and regenerative properties, providing an attractive therapeutic approach for pulmonary arterial hypertension (PAH)[1]. Despite the therapeutic potential of MSCs for improving the outcomes of PAH patients[2,3], no more than 5% of cells survive after transplant[4]. Thus, the low survival rate of the grafted cells is widely perceived as the major hindrance for an MSC-based therapy for PAH.

Anoikis occurs when cells detach from the extracellular matrix and subsequently undergo apoptosis, and potentially acts as a major enabling factor for the apoptosis of transplanted cells[5,6]. Indeed, after isolation from the extracellular matrix (ECM) and injection into the circulatory system for transplantation, MSCs will undergo anoikis, also referred to as cell isolation-induced apoptosis, leading to a series of alterations in anoikis signalling pathways[3,7,8]. Anoikis can be induced by destruction of integrin (ITGs) signalling or deletion of ITGs genes[9]. After isolation, focal adhesion kinase (FAK), a key downstream target of ITGs, is recruited to focal adhesion sites, consequently activating cell survival signals, such as blocking caspase 3 expression[10]. However, it remains unknown whether ITGs signalling is involved in the process of anoikis in MSCs.

Autophagy is a dynamic process that maintains homeostasis by preventing the accumulation of excessive biomolecules and impaired cells and organelles. There is accumulating evidence of a link between autophagy and anoikis[11]. Previously, we demonstrated that mammalian Ste20-like kinase 1 (Mst1) inhibition could reduce H_2O_2-induced apoptosis of mBMSCs by inducing autophagy formation [12]. Mst1 is a serine/threonine kinase, known as a key mediator in cellular processes, including mediating the apoptosis[13]. However, the molecular mechanism by which Mst1 inhibition mediates autophagy and anoikis in isolated mBMSCs remains to be clarified.

In this study, we investigated the potential regulatory effect of Mst1 inhibition on ITGs signalling, autophagy and anoikis in isolated mBMSCs.

MATERIALS AND METHODS

Cell culture

The mBMSCs were obtained as previously described[12]. Cultured mBMSCs between passages 3 and 5 were selected for subsequent experiments.

Adenovirus infection

Adenovirus harbouring Mst1 short hair RNA (Ad-sh-Mst1) and the control vector for Mst1 shRNA (Ad-NC-Mst1) were purchased from WZ Biosciences (China). Vector details have been previously described [12]. The shRNA sequence targeting Mst1 in mice was GCCCTCACGTA GTCAAGTATT.

siRNA transfection

The small interfering RNAs (siRNAs) were obtained from GenePharma (China). The sense and antisense strand sequences of siRNA are as follows: Mouse siRNA-ITGα5, 5'-GCAGGGAGA-UGAAGAUCUACC' (sense) and 5'-UAGAUCUUCAUCUCCCUGCAG' (antisense); mouse siRNA-ITGβ 1, 5'-GGAGAACCACAGAAGUUUACA-3' (sense) and 5'-UAAACUUCUGUGGUUCUCCUG-3' (antisense); and siRNA-negative control (NC), 5'-UUCUCCGAACGUGUCACGUTT' (sense) and 5'-ACGUGACACGUUCGGAGAATT-3' (antisense). Subsequently, 24 h after infection with Ad-sh-Mst1, mBMSCs at 75% confluence were transfected with ITGα5, ITGβ1 or NC siRNA (50 nM) using Lipofectamine RNAi MAX (13778500, Invitrogen) according to the manufacturer's instructions. The expression of ITGα5 or ITGβ1 was substantially blocked by the transfected siRNA.

Cell treatment

Petri dishes coated with polyhydroxyethyl methacrylate [Poly-HEMA, 529257, Sigma, United States of America (USA)] were used to prevent cells from adhering to the tissue culture plates. Briefly, poly-HEMA stock material was dissolved in 95% ethanol at a concentration of 12 mg/mL, and 1 mL of 12 mg/mL poly-HEMA was added to each well of a 6-well plate and then dried overnight on a clean bench. Cells were transfected as previously described. Cells (5×10^5) were coated with 12.5 mg/mL poly-HEMA in each well for a certain period of time.

To inhibit autophagy, cells were pretreated with 5 mmol/L 3-MA (189490, Selleck, USA) for 1 h and then cultured in poly-HEMA-precoated plates for a certain period of time.

Assay of intracellular ROS

As mentioned above, cellular ROS were assessed using the ROS probe 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA), S0033, Beyotime Biotechnology, China)[12]. The mean fluorescence intensity was detected *via* flow cytometry.

Isolation-induced anoikis assay

Anoikis was analysed using an in situ Direct DNA Fragmentation Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) Assay Kit [ab66108, Abcam, The United Kingdom of Great Britain and Northern Ireland (UK)]. After incubation in poly-HEMA-coated plates, the cells were collected and added to 70% ice ethanol for 30 min. Ethanol was then removed, and the cells were resuspended in washing buffer and then stained with a staining solution for 60 min. Prior to the addition of the PI/RNase A solution, the cells were washed twice with rinse buffer. Quantification analysis was performed by BectonDickinson Fluorescence Activating Cell Sorter (BD FACSDiva) software, [Ex/Em = 488/520 nm for fluoresceine isothiocyanate and 488/623 nm for propidium iodide)].

Anoikis was also detected by a CytoSelect™ 24-Well Anoikis Assay (XY-CBA-080, Cell Biolabs, USA) according to the manufacturer's instructions. Briefly, cells (1×10^6 cells/well) were cultured in each well of 24-well plate for 36 h before staining with ethidium homodimer (EthD-1) at 37°C for 1 h. The presence of red EthD-1 fluorescence in dead cells was observed by a fluorescence microscope, and cell viability was determined using a thiazolyl blue tetrazolium bromide (MTT) assay.

Flow cytometry

Cells were incubated in poly-HEMA-coated petri dishes for 36 h, centrifuged at 300 × *g* for 5 min and cultured in antibodies (ITGα4 [1/500 dilution, 553157, BD], ITGα5 [1/500 dilution, 557447, BD], ITGαv [1/300 dilution, 740946, BD], ITGβ1 [1/500 dilution, 561796, BD], ITGβ3 [1/100 dilution, 740677, BD]) for 1 h according to the operation manual.

Cell adhesion

After culture in poly-HEMA-coated petri dishes, the collected cells were resuspended in complete α-MEM and then plated in triplicate (5×10^4 cells/well) onto wells coated with fibronectin (10 g/mL), which was previously blocked with 1% BSA for 1 h. After 6 h, the cells were washed with phosphate belanced solution (PBS) and stained with crystal violet. Unbound dye was removed with PBS before adding a 10% acetic acid solution. The absorbance was read at 630 nm using a Multiskan MK3 microplate reader. The experiment was repeated three times. Cell adhesion was calculated according to the proportion of adhered cells in the control group.

Cytokine levels

The supernatants in each group were collected after culture in poly-HEMA-coated petri dishes for 36 h. The levels of anti-inflammatory cytokines were measured using a BD™ Cytometric Bead Array (CBA)

Mouse Th1/Th2/Th17 Cytokine Kit (561665, BD, USA) in accordance with the instruction manual. The levels of interleukin (IL)-4 (IL-4), IL-10, IL-17A and IL-6 in cell supernatants were measured using flow cytometry. Data analysis was performed as previously described[14].

Nude mouse tumorigenicity

All animal procedures were approved by the Animal Care and Use Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University (IACUC protocol, Approval No. 2020-333). A total of 10 female nude mice (4 wk old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and raised in a specific pathogen-free environment. Mice were placed at a standard room temperature at a normal day-night cycle with free access to standard diet and water. Afterwards, 5.0 × 10^6 mBMSCs (n = 3), mBMSC/NC-Mst1 (n = 3), and mBMSC/sh-Mst1 (n = 4) were injected into the right flank near the hind legs of each nude mouse. The tumours were measured with a Vernier calliper every 4 d. Sixty days after cell inoculation, all mice were anaesthetized with ether, and tissues were collected.

Quantitative real-time polymerase chain reaction (qPCR)

qPCR was performed as previously reported[12]. mBMSCs were differentiated *via* 21-d exposure to osteogenic or adipogenic conditions, and total mRNA from mBMSCs subjected to these conditions and siRNA-transfected cells was isolated using TRIzol Reagent (15596026, Thermo Fisher Scientific, USA). The RNA was subsequently reverse transcribed into cDNA and amplified using the SYBR® Premix Ex Taq™ II kit (RR420, Takara, JPN) and d ABI 7500 real-time PCR system (Applied Biosystems). Each experiment was repeated three times. Data were normalized through the $2^{-\Delta\Delta CT}$ method using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The primer sequences are shown in Supporting Information Supplementary Table 1.

Western blot analysis

To determine protein expression, Western blot analysis was performed. After culture in poly-HEMA-coated plates, whole-cell protein extracts were prepared in radio-immunoprecipitation assay lysis buffer, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene fluoride membranes. The membranes were then blocked with 5% skimmed milk or Bovine serum albumin in Tris-Buffered Saline Tween-20 for 1 h and incubated overnight at 4 °C with the following primary antibodies (diluted by Western Primary Antibody Buffer, P0023A, Beyotime): Mst1 (1:1000, ab51134, Abcam), ITGα5 (1:1000, ab150361, Abcam), ITGβ1 (1:1000, ab179471, Abcam), phospho-FAK (Tyr397) [1:500, 3283S, Cell Signaling Technology (CST)], FAK (1:1,000, 3285S, CST), activated caspase 3 (1:1000, ab214430, Abcam), and caspase 3 (1:1000, ab18297, Abcam). GAPDH (1:1000, 5174S, CST) served as the loading control. Anti-rabbit IgG and HRP-linked antibodies (1:1000, 7074S, CST) were used. The relative protein expression levels were compared with GAPDH using ImageJ software.

Statistical analysis

All results are expressed as the mean ± SD. One-way Analysis of Variance was used for data analysis. $P < 0.05$ was considered statistically significant.

RESULTS

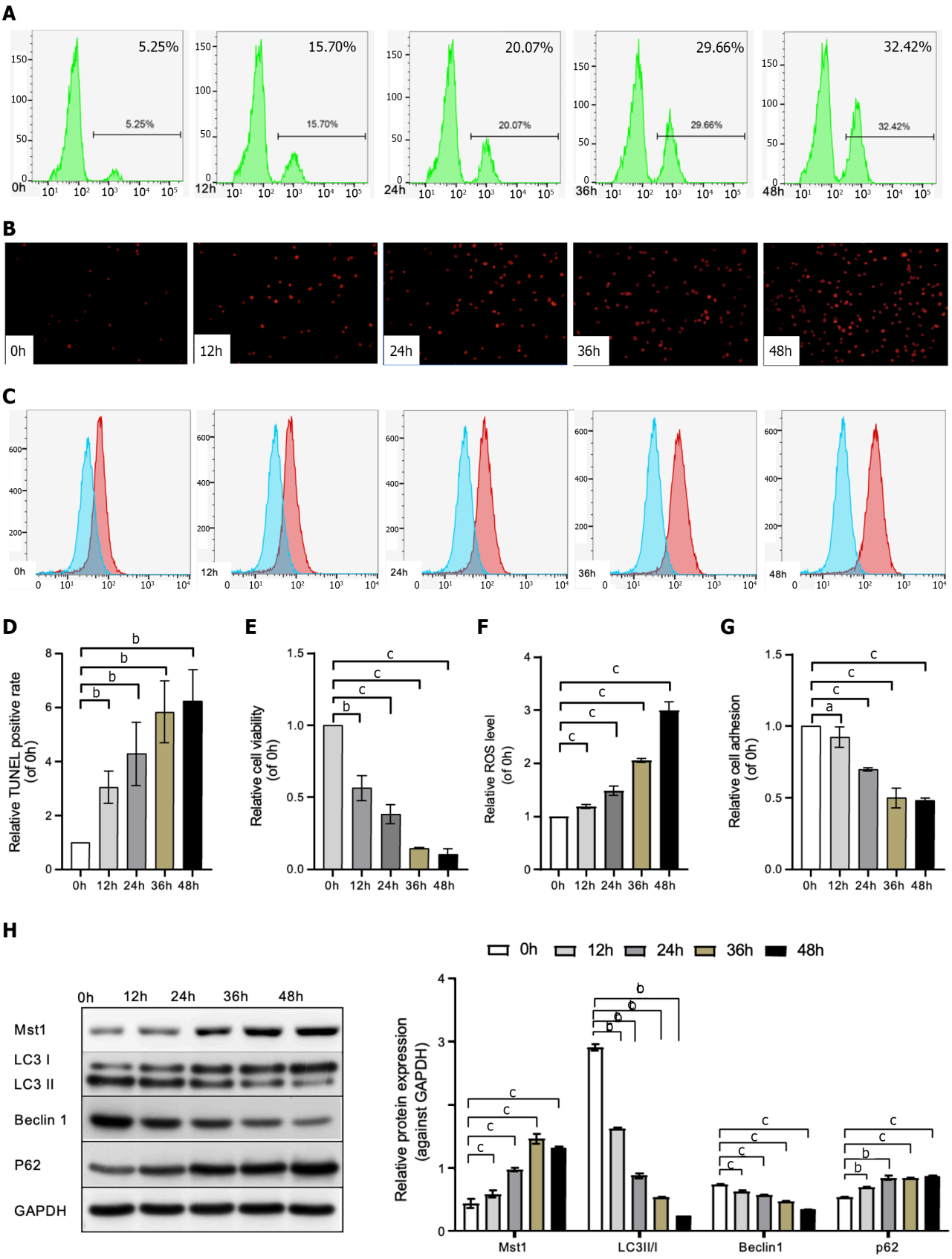
The loss of attachment to ECM increased the rate of aberrant cell apoptosis, ROS levels, and Mst1 expression and inhibited autophagy in mBMSCs

As the ability to reduce cell adhesivity to culture plates, Poly-HEMA was used to simulate an anchorage-independent culture condition. In present study, the sensitivity of mBMSCs to anoikis in Poly-HEMA-pre-coated condition were tested.

Using the TUNEL and Anoikis Assay Kit, the results showed an increased rate of mBMSC apoptosis in a time-dependent manner under poly-HEMA-induced isolated conditions (Figures 1A, B, D and E), suggesting that anoikis of mBMSCs could be induced in precoated poly-HEMA plates. In addition, the cell adhesion decreased at 24 h, 36 h, and 48 h compared with that at 0 h (Figure 1G).

Moreover, staining of intracellular ROS with the ROS probe DCFH-DA showed increased ROS levels at 24, 36, and 48 h compared with 0 h (Figures 1C and F), demonstrating the production of ROS in poly-HEMA-induced isolated mBMSCs.

To determine the alterations in Mst1 expression, autophagy and the FAK/Caspase 3 pathway in mBMSCs under isolated conditions, the protein level of Mst1, autophagy-related proteins (LC3 II/I, Beclin1, p62), p-FAK, and activated caspase 3 was detected by Western blot analysis. The data suggested that Mst1 was upregulated in isolated mBMSCs (Figure 1H). Moreover, the expression of p-FAK decreased, and the activation of caspase 3 increased in a time-dependent manner (Figure 1I). Similarly, LC3 II/I and Beclin1 expression was downregulated, and p62 expression was upregulated in a time-dependent manner (Figure 1H).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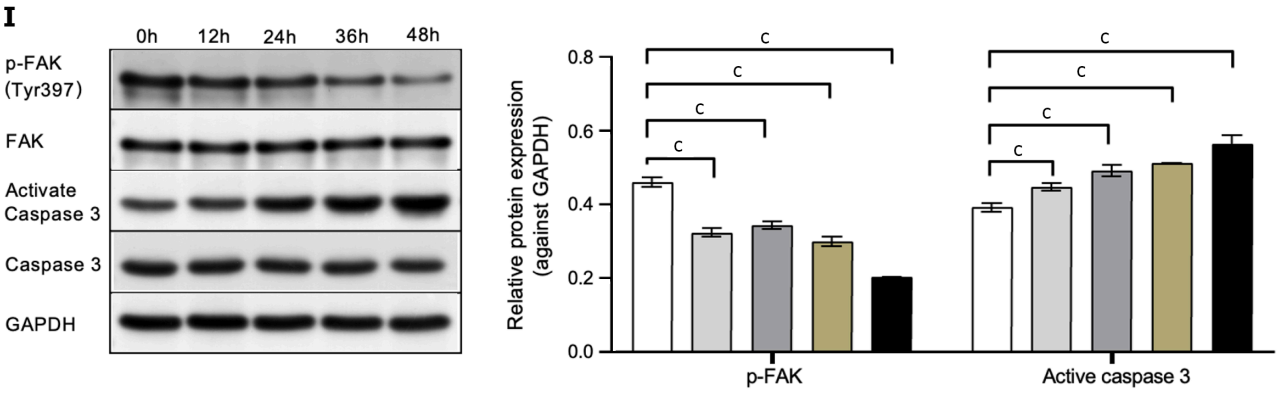

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Figure 1 Mouse bone marrow mesenchymal stem cells exhibit susceptibility to anoikis under detachment conditions. A: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL)-positive cells (apoptotic cells) of mouse bone marrow mesenchymal stem cells (mBMSCs) cultured in precoated Poly-2-hydroxyethyl methacrylate conditions for 0 h, 12 h, 24 h, 36 h and 48 h using flow cytometry; B: Fluorescence staining images of dead cells; C: Reactive oxygen species (ROS) levels were measured using the ROS probe 2,7-Dichlorodihydrofluorescein diacetate by flow cytometry at 0 h, 12 h, 24 h, 36 h and 48 h; D: Quantitative analysis of the rate of TUNEL-positive cells; E: Quantification of live cells using a thiazolyl blue tetrazolium bromide assay; F: Quantitative analysis of the intracellular ROS level; G: Cell adhesion was expressed as fold changes between 0 h groups; H and I: Mammalian Ste20-like kinase 1 (Mst1), microtubules associated protein 1 light chain 3 II/I, p62, Beclin1, phospho-focal adhesion kinase, and activated caspase 3 expression levels at 0 h, 12 h, 24 h, 36 h and 48 h were evaluated by Western blot analysis; The expression of protein was expressed as the fold change relative to glyceraldehyde-3-phosphate dehydrogenase. Values are the mean ± SD of three independent experiments in each case. TUNEL: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling; ROS: Reactive oxygen species; Mst1: Mammalian Ste20-like kinase 1; LC3-II/I: Microtubules associated protein 1 light chain 3 II/I; p-FAK: Phospho-focal adhesion kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. $^{c}P < 0.01$; $^{b}P < 0.05$; $^{a}P > 0.05$.

Mst1 inhibition upregulated ITGα5 and ITGβ1 expression in isolated mBMSCs

The mBMSCs were infected with adenovirus containing Mst1 shRNA. The effect of shRNA on inhibiting Mst1 expression were measured by qPCR and Western blot (Supplementary Figure 1).

Evidence has shown that ITGs, the heterodimeric cell surface adhesion receptors, mediates anoikis. In this study, the alterations of ITGs in isolated mBMSCs/sh-Mst1 were tested.

The expression profiles of ITGα5, ITGαv, ITGα4, ITGβ1, and ITGβ3 in poly-HEMA-treated mBMSCs were compared by flow cytometry. Compared with the control mBMSC levels, the poly-HEMA-treated isolated mBMSC levels of ITGα5, ITGαv, ITGα4, ITGβ1, and ITGβ3 were significantly decreased (Figure 2). Compared with isolated mBMSCs, isolated mBMSCs/sh-Mst1 show an upwards trend in ITGα5 and ITGβ1 expression. However, there was no difference of the expression profiles of ITGαv, ITG α4, and ITGβ3 between isolated mBMSCs and isolated mBMSCs/sh-Mst1 (Figure 2). This study suggested that the inhibition of Mst1 could reactivate the expression of ITGα5 and ITGβ1.

Suppression of Mst1 expression protected mBMSCs from anoikis by activating autophagy.

mBMSCs were cultured in precoated poly-HEMA plates for 36 h. A significant decrease in cell apoptosis was observed in mBMSCs/sh-Mst1 (Figures 3A, B, D and E). Similar to the above results, cell adhesion was ameliorated by silencing Mst1 expression (Figure 3G). These results indicated that Mst1 inhibition suppressed ECM-isolatedinduced anoikis in mBMSCs.

In addition, flow cytometric analysis confirmed decreased ROS levels in isolated mBMSCs/sh-Mst1 compared with those of isolated mBMSCs, whereas ROS levels were re-elevated by the autophagy inhibitor 3-MA (Figures 3C and F).

Western blot assay further suggested the above conception. FAK, has been recognised as the key mediator of cell–substrate adhesion. Western blot analysis results showed that mBMSC/sh-Mst1 exhibited robust FAK activation (Figure 3I). Similar to apoptosis, the activation of caspase can induce anoikis. Thus, we tested effect of Mst1 inhibition on the activation of caspase 3 by Western blotting. In Figure 3I, silencing Mst1 expression significantly inhibited caspase 3 activation in suspension-grown mBMSCs. This study indicated that silencing Mst1 expression could reactivate the FAK/Caspase3 pathway in anchorage-independent mBMSCs. However, 3-MA, an autophagy inhibitor, had no effect on the expression of ITGα5 and ITGβ1 or on cell adhesion (Figure 3I).

Consistent with the previous results, Mst1 inhibition reactivated autophagy in mBMSCs under isolated conditions, which can be demonstrated by the upregulated expression of LC3 II/I and Beclin1 and downregulated expression of p62 (Figure 3H). Furthermore, the number of mBMSCs/sh-Mst1 undergoing anoikis was increased after pretreatment with 3-MA (Figure 3H). In conclusion, the protective effect of Mst1 knockdown on anoikis in mBMSCs is associated with autophagy.

Inhibition of ITGα5β1 reversed the protective effects of Mst1 inhibition against anoikis in mBMSCs

To determine whether ITGα5 or ITGβ1 contributes to anoikis resistance in mBMSC/sh-Mst1 cells,

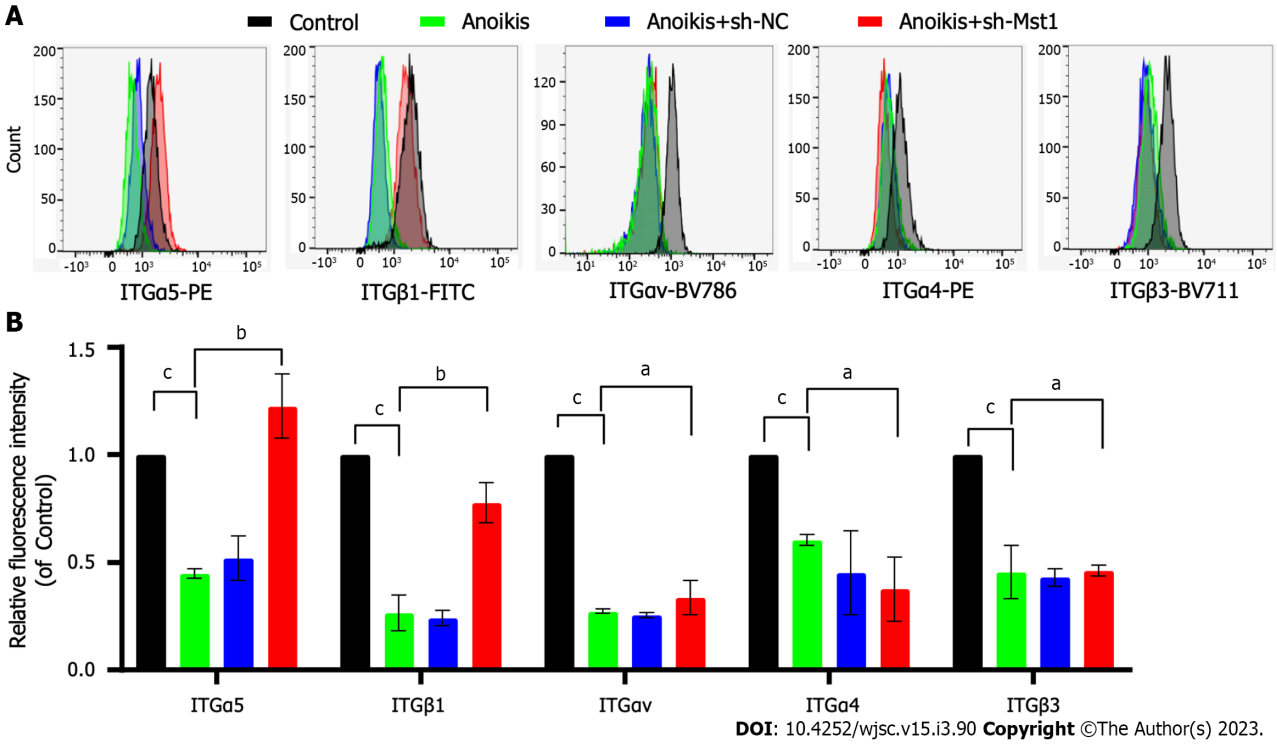

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Figure 2 Changes in integrin protein expression in detached mouse bone marrow mesenchymal stem cells/sh-Mammalian Ste20-like kinase 1. Cells were cultured in detached conditions for 36 h. A: Flow cytometry analysis investigating the expression of integrinα5 (ITGα5), ITGαv, ITGα4, ITGβ1 and ITGβ3; B: Data are expressed as the fold change compared to the control groups. Values are expressed as the mean ± SD, $n = 3$. ITG: Integrin. $^{c}P < 0.01$; $^{b}P < 0.05$; $^{a}P > 0.05$.

siRNA was used to knock down ITGα5 or ITGβ1 expression, respectively (Supplementary Figure 2).

In isolated mBMSC/sh-Mst1, cell apoptosis was increased, and cell adhesion was blocked by siRNA-mediated ablation of ITGα5 or ITGβ1 (Figures 4A, B, D, E and G). Similarly, p-FAK expression was downregulated and caspase3 activation was upregulated using ITGα5 or ITGβ1 siRNA (Figure 4I). In addition, LC3 II/I, Beclin1 and p62 expression was also reversed by ITGα5 or ITGβ1 siRNA (Figure 4H). In addition, the results in Figures 4C and F suggested that the ROS level was reduced by ITGα5 or ITGβ1 siRNA.

The properties and biological safety of mBMSCs/sh-Mst1

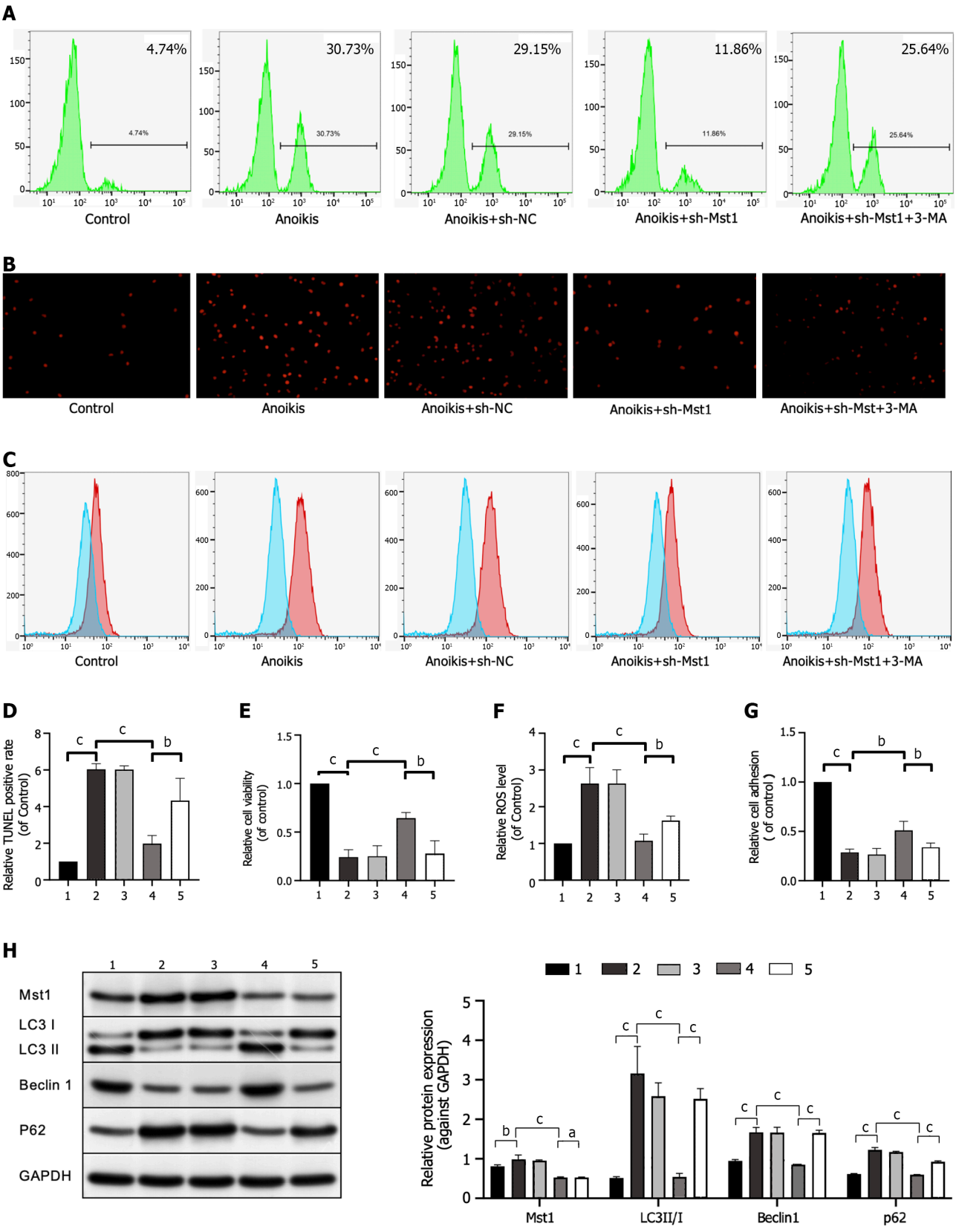
In isolated conditions, the levels of anti-inflammatory cytokines IL-4, IL-10 and IL-17A increased, while the level of pro-inflammatory cytokine IL-6 decreased in mBMSCs/sh-Mst1 compared with those of other mBMSCs (Figure 5A).

We assessed the effect of silencing Mst1 expression on the osteogenic differentiation of mBMSCs. In Figure 5B, Mst1 inhibition was correlated with increased osteogenic differentiation of mBMSCs. Subsequently, qPCR was performed to accurately determine the role of Mst1 inhibition on osteogenic differentiation in mBMSCs. As known as the markers of osteoblast differentiation, we tested the mRNA levels of runt-related transcription factor 2 (Runx2) and alkaline phosphatase (ALP). We found that the expression of Runx2 and ALP were both increased in mBMSC/sh-Mst1 (Figure 5B)[15].

There was no tumour-like mass in animals injected with mBMSC/sh-Mst1 after 60 d post-injection. After 60 d post-injection, we collected the subcutaneous tissue and the lung, liver, kidney and heart. There were no difference of the weights of the lung, liver, kidney and heart among each group (Figures 5C and Supplementary Table 2). It also showed that no stromal structures appeared in subcutaneous tissue of mBMSCs/sh-Mst1 groups (Figure 5C).

DISCUSSION

Convincing suggestion has confirmed that anoikis limits the therapeutic efficacy of MSC transplantation for tissue repair[16]. Herein, this study has proven that mBMSC/sh-Mst1 could survive after isolation from the ECM, and this response was mediated by the effect of Mst1 inhibition-induced autophagy on ITGα5β1-modulated production of ROS.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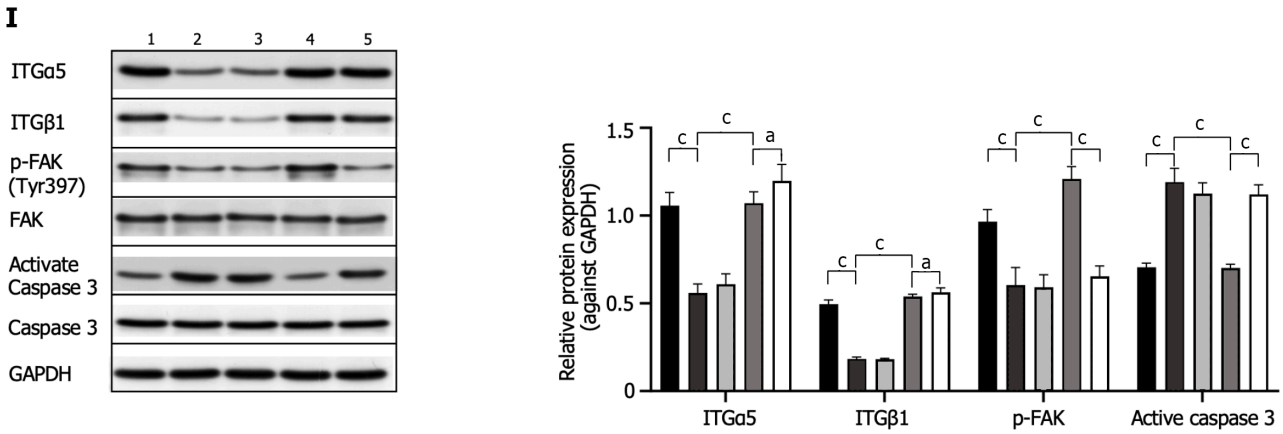

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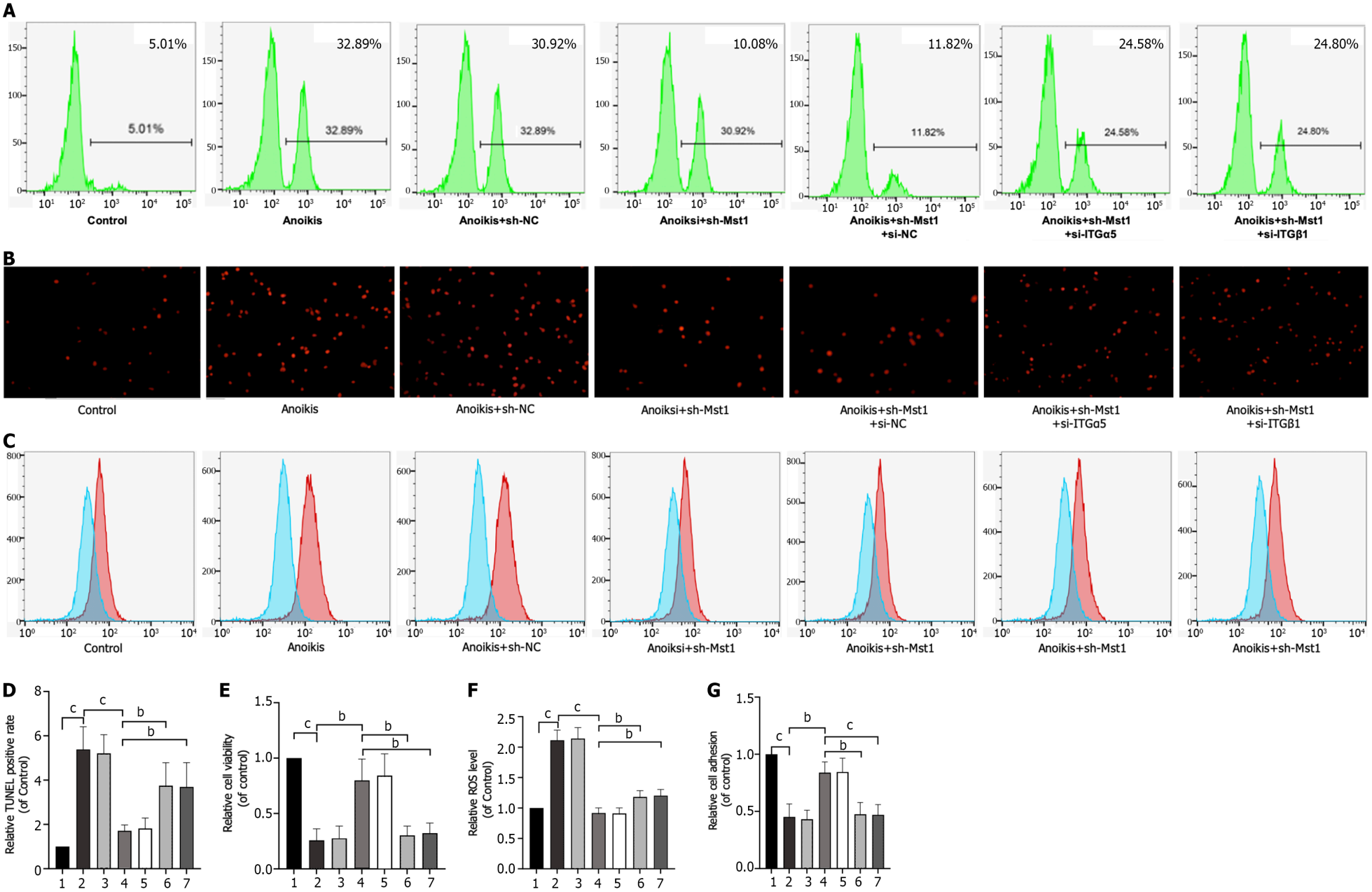
Figure 3 Mammalian Ste20-like kinase 1 inhibition-induced autophagy reduced cell apoptosis in detached mouse bone marrow mesenchymal stem cells. A: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL)-positive cells analysed by flow cytometry; B: Representative images and quantification of fluorescence staining of dead cells; C: Reactive oxygen species (ROS) levels were measured using the ROS probe 2,7-Dichlorodihydrofluorescein diacetate by flow cytometry. D: Quantitative analysis of the rate of TUNEL-positive cells. E: Quantification of live cells using a thiazolyl blue tetrazolium bromide assay. F: Quantitative analysis of the intracellular ROS level. G: Cell adhesion was evaluated as the fold change compared back to the control groups; H and I: Mammalian Ste20-like kinase 1 (Mst1), microtubules associated protein 1 light chain 3 II/I, p62, Beclin1, phospho-focal adhesion kinase, and activated caspase 3 expression were evaluated by Western blot analysis. glyceraldehyde-3-phosphate dehydrogenase served as a control. Values are the mean ± SD, and experiments were completed in triplicate. 3-MA: 3-methyladenine; TUNEL: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling; ROS: Reactive oxygen species; Mst1: Mammalian Ste20-like kinase 1; LC3-II/I: Microtubules associated protein 1 light chain 3 II/I; p-FAK: Phospho-focal adhesion kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. 1. Control group; 2. Anoikis group; 3. Anoikis + sh-NC group; 4. Anoikis + sh-Mst1 group; 5. Anoikis + sh-Mst1 + 3-MA group. $^{c}P < 0.01$; $^{b}P < 0.05$; $^{a}P > 0.05$.

Corresponding alterations in cell-ECM isolation and autophagy also exist[17]. As a special type of apoptotic cell death, anoikis contributes to the loss of cell attachment to the ECM[18,19]. In the present study, we observed increased cell apoptosis and inhibited autophagy, as well as upregulated Mst1 expression in isolated mBMSCs. One hypothesis derived from a combination of previous studies is that Mst1 inhibition can not only overcome anoikis but also induce autophagy in isolated mBMSCs. In this study, we confirmed that mBMSCs averted anoikis by Mst1 inhibition-induced autophagy. Autophagy promotes cell survival or apoptosis in a stimulus-dependent manner. A series of experiments have elucidated the role of autophagy in promoting cell survival during anoikis[20]. Accordingly, our results established Mst1 inhibition-induced autophagy as a survival mechanism in isolated mBMSCs.

ITGs are transmembrane αβ heterodimers, with at least 18 well-known α and 8 β subunits. An increasing amount of experimental data has demonstrated that cells can overcome anoikis by changing ITGs expression[21]. In addition, ITGs-mediated cell adhesion to ECM is critical for maintaining appropriate cellular function and survival[22]. Therefore, the upregulation of ITGs allows cells to survive during anoikis[9,22,23]. This study has proved that the expression of ITGα5 and ITGβ1 were increased in cultured mBMSCs/sh-Mst1 under cell isolation conditions. Furthermore, upregulated ITGα 5 and ITGβ1 expression may be the underlying mechanism of anoikis resistance in mBMSCs/sh-Mst1. These results suggested the role of ITGα5β1 downstream of Mst1, as well as a collaboration between ITG α5 and ITGβ1, in anoikis-resistant mBMSCs/sh-Mst1.

ITGs relay signals from the ECM to initiate intracellular signalling through intracellular ROS production[24], by which p-FAK expression is mediated[25]. Moreover, a recent study confirmed that excessive or persistent increases in ROS levels might promote the process of anoikis[26]. However, high ROS levels may also promote the formation of autophagy, which could contribute to reducing ROS accumulation[27]. Despite the essential role of increased ROS levels in anoikis resistance reported in several studies[28], we still hypothesized the necessity of appropriate cellular regulation of ROS levels for anoikis inhibition. As a result, we speculated that a negative-feedback loop was formed among Mst1 inhibition-induced autophagy, Mst1 inhibition-triggered ITGα5β1 and ROS levels. Mst1 inhibition increased ITGα5β1 expression, thereby facilitating cell adhesion. In addition, Mst1 inhibition-induced autophagy reduced the level of ITGα5β1-triggered ROS in isolated mBMSCs, which contributed to the evasion of anoikis, elucidating why 3-MA did not affect the expression of ITGα5 or ITGβ1.

Mst1 has been known to play a key role in the signalling pathway that controls manifold cellular processes[29]. In the present study, silencing Mst1 expression was found to ameliorate the anti-inflammatory cytokine production, osteogenic differentiation capability and cell proliferation of mBMSCs, thereby making mBMSCs/sh-Mst1 an attractive target for anti-inflammatory, immunomodulatory, and regenerative therapies and potentially improving the curative efficacy of mBMSCs in PAH[1-3].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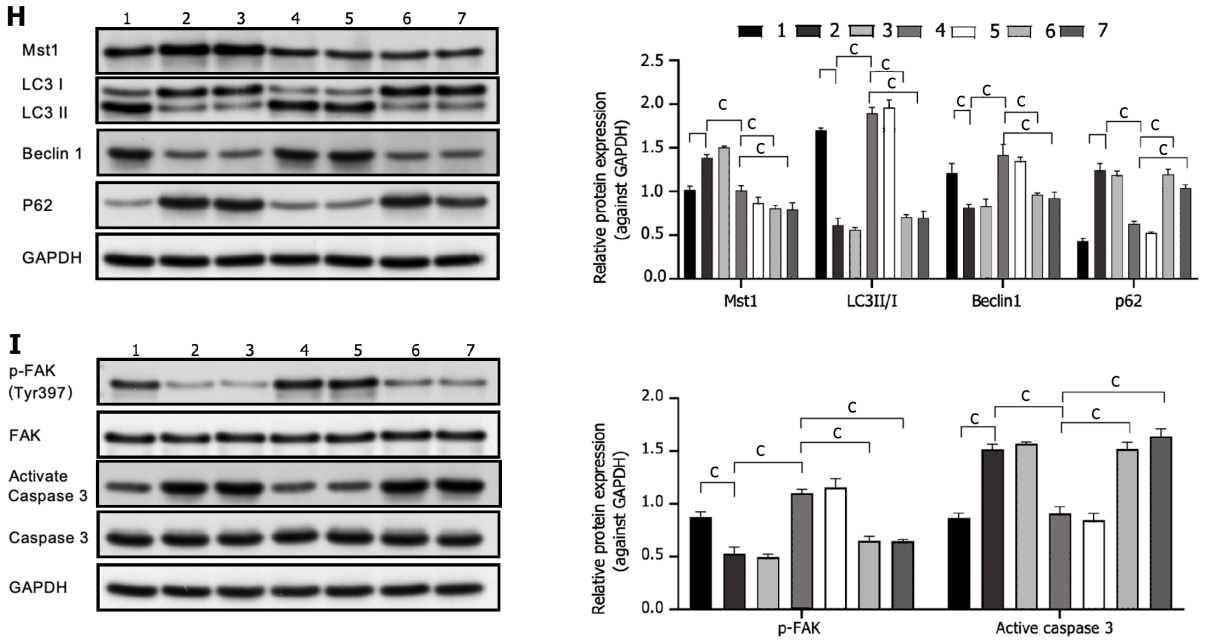

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Figure 4 Upregulated integrinα5β1 expression triggered by Mammalian Ste20-like kinase 1 inhibition protected mouse bone marrow mesenchymal stem cells from anoikis. A: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL)-positive cells analysed by flow cytometry; B: Representative images and quantification of fluorescence staining of dead cells; C: Reactive oxygen species (ROS) levels were measured by flow cytometry; D: Quantitative analysis of the rate of TUNEL-positive cells; E: Quantification of live cells using a thiazolyl blue tetrazolium bromide assay; F: Quantitative analysis of the intracellular ROS level; G: Cell adhesion was evaluated as the fold change compared back to the control groups; H and I: Western blotting was used to measure the expression levels of Mammalian Ste20-like kinase 1 (Mst1), microtubules associated protein 1 light chain 3 II/I, p62, Beclin1, phospho-focal adhesion kinase, and activated caspase 3. glyceraldehyde-3-phosphate dehydrogenase was used as the loading control. Values are expressed as the mean ± SD. Measurements were performed in three replicates. 3-MA: 3-methyladenine; TUNEL: Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling; ROS: Reactive oxygen species; Mst1: Mammalian Ste20-like kinase 1; LC3-II/I: Microtubules associated protein 1 light chain 3 II/I; p-FAK: Phospho-focal adhesion kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. 1. Control group; 2. Anoikis group; 3. Anoikis + sh-NC group; 4. Anoikis + sh-Mst1 group; 5. Anoikis + sh-Mst1 + si-NC group; 6. Anoikis + sh-Mst1 + si-ITGα5; 7. Anoikis + sh-Mst1 + si-ITGβ1. $^{c}P < 0.01$; $^{b}P < 0.05$; $^{a}P > 0.05$.

Regardless of the extraordinary safety profile of MSC therapy verified in clinical trial data, several scholarly reviews have proposed that MSCs play a role in tumorigenesis and progression[18,30,31]. Therefore, the enhancement of the anti-anoikis ability of MSCs may promote tumorigenesis. However, in the present study, tumorigenic experiments in nude mice demonstrated the safety profile of mBMSC/sh-Mst1 administration.

CONCLUSION

In summary, the mechanism by which Mst1 inhibition acts on anoikis in mBMSCs was expounded in

this study. First, Mst1 inhibition was demonstrated to ameliorate not only autophagy formation but also ITGα5β1 expression. Second, Mst1 inhibition-induced autophagy could scavenge the excessive production of ITGα5β1-triggered ROS. Third, silencing Mst1 expression not only ameliorated the pluripotency of mBMSCs but also retained the safety profile of mBMSCs. Overall, Mst1 inhibition-based infusion may improve the therapeutic efficacy of MSCs, thereby serving as the ideal candidate for clinical transplant therapy in PAH.

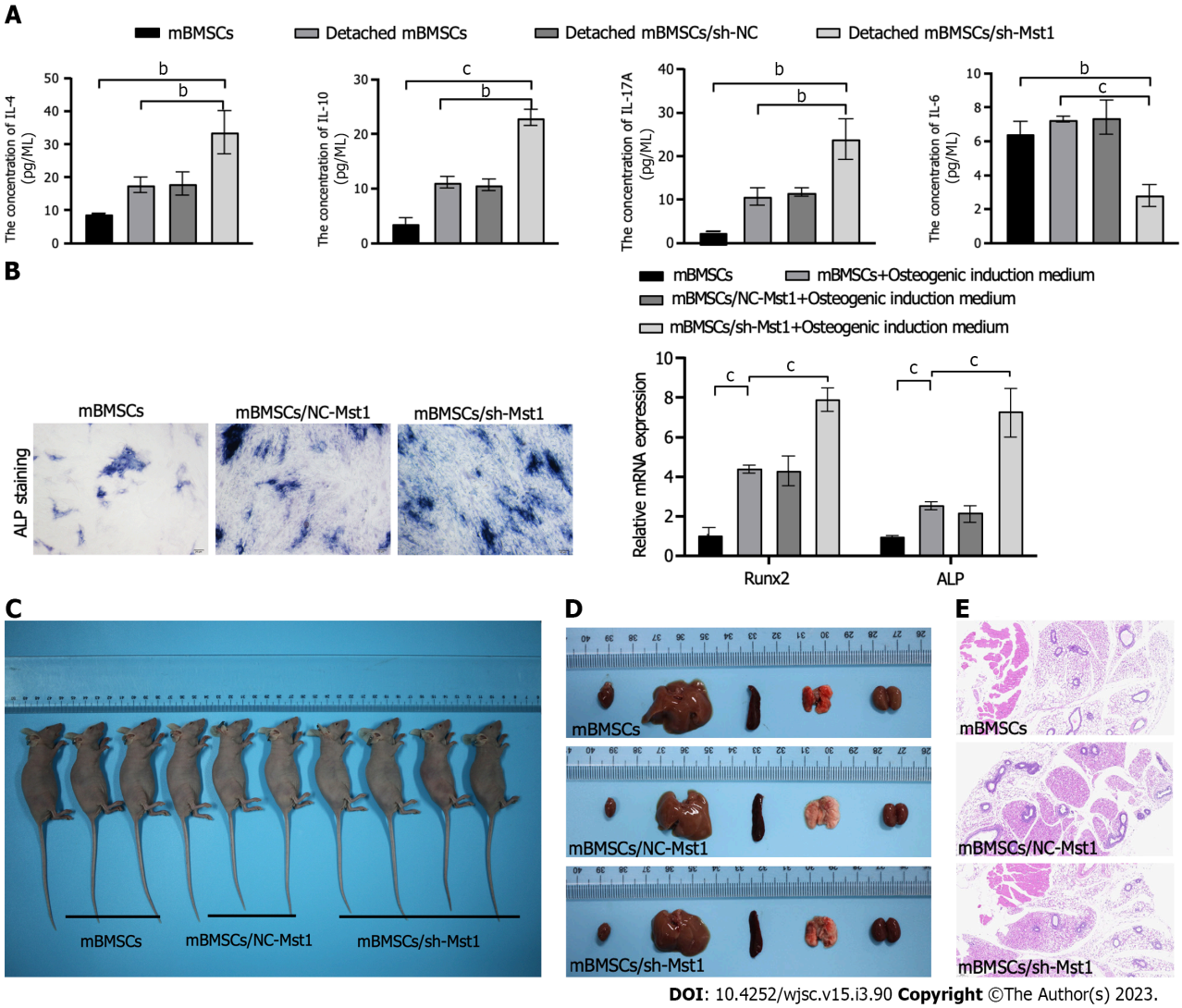

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Figure 5 The properties and biological safety of mouse bone marrow mesenchymal stem cells/sh-Mammalian Ste20-like kinase 1. A: The levels of interleukin-4 (IL-4), IL-10, IL-17A and IL-6 were measured using a Cytometric Bead Array Cytokine Kit in detached mouse bone marrow mesenchymal stem cells (mBMSCs); B: Representative images of alkaline phosphatase (ALP) staining of mBMSCs after culture in osteogenesis induction medium for 21 d; C: Quantitative real-time polymerase chain reaction analysis of the relative mRNA expression levels of runt-related transcription factor 2 (Runx2) and ALP in mBMSCs cultured in adipogenesis induction medium for 21 d; D: Nude mice were subcutaneously implanted with mBMSCs (n = 3), mBMSCs/NC-Mst1 (n = 3), and mBMSCs/sh-Mst1 (n = 4). Representative images of the heart, liver, spleen, lung and kidney in each group 60 d postinjection; E. Paraffin-embedded subcutaneous sections derived from cell-injection sites were stained with haematoxylin and eosin. Data are the mean ± SD of three technical replicates. mBMSCs: Mouse bone marrow mesenchymal stem cells; Mst1: Mammalian Ste20-like kinase 1; IL: Interleukin; Runx2: Runt-related transcription factor 2; ALP: Alkaline phosphatase. $^{c}P < 0.01$; $^{b}P < 0.05$; $^{a}P > 0.05$.

ARTICLE HIGHLIGHTS

Research background

Anoikis plays a limiting role in the therapeutic efficacy of mesenchymal stem cells (MSCs). As a proapoptotic molecule, mammalian Ste20-like kinase 1 (Mst1) can increase the production of reactive oxygen species (ROS), thereby promoting anoikis. Recently, Mst1 inhibition was found to protect mouse bone marrow MSCs (mBMSCs) from H_2O_2-induced cell apoptosis by inducing autophagy and reducing ROS production. However, the influence of Mst1 inhibition on anoikis in mBMSCs remains unclear.

Research motivation

To investigate whether Mst1 inhibition could reduce anoikis in isolated mBMSCs.

Research objectives

To investigate the mechanisms by which Mst1 inhibition acts on anoikis in isolated mBMSCs.

Research methods

Poly-2-hydroxyethyl methacrylate-induced anoikis was used following Mst1 inhibition in mBMSCs. Integrin (ITGs) levels were tested by flow cytometry. Autophagy and ITGα5β1 were inhibited using 3-methyladenine and small interfering RNA, respectively. The alterations in anoikis were evaluated by Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling and anoikis assays. The levels of the anoikis-related proteins ITGα5, ITGβ1, and phospho-focal adhesion kinase, which activate caspase 3, and the autophagy-related proteins microtubules associated protein 1 light chain 3 II/I, Beclin1 and p62 were detected by Western blotting.

Research results

In isolated mBMSCs, Mst1 expression was upregulated, and Mst1 inhibition significantly reduced cell apoptosis, induced autophagy and decreased ROS levels. Mechanistically, we found that Mst1 inhibition upregulated ITGα5 and ITGβ1 expression but not ITGα4, ITGαv, or ITGβ3 expression. Moreover, ITGα5β1 upregulation and autophagy induction by Mst1 inhibition played an essential role in terms of the protective efficacy of Mst1 inhibition on averting anoikis.

Research conclusions

Mst1 inhibition ameliorated autophagy formation, increased ITGα5β1 expression, and decreased the excessive production of ROS, thereby reducing cell apoptosis in isolated mBMSCs. On this basis, Mst1 inhibition may provide a promising strategy to overcome the anoikis of transplanted MSCs.

Research perspectives

In isolated mBMSCs, Mst1 inhibition ameliorated not only autophagy formation but also ITGα5β1 expression (not ITGα4, ITGαv, or ITGβ3). Mst1 inhibition-induced autophagy scavenged excessive ITGα5β1-triggered ROS. Consequently, Mst1 inhibition-based infusion may improve the therapeutic efficacy of MSCs, thereby serving as an ideal candidate for clinical transplantation in pulmonary arterial hypertension.

FOOTNOTES

Author contributions: Yu WC contributed to the conception and design and manuscript writing; Zhang T contributed to conception and design, collection and assembly of data, data analysis and interpretation. Yu WC, Zhang T, Zhang Q performed the experiments; All authors participated in discussing, revising the manuscript, and approving the final manuscript; All authors have read and approve the final manuscript.

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Conflict-of-interest statement: The authors declare that they have no conflicts of interest with the contents of this article.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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REFERENCES

1 **Bari E**, Ferrarotti I, Torre ML, Corsico AG, Perteghella S. Mesenchymal stem/stromal cell secretome for lung regeneration: The long way through "pharmaceuticalization" for the best formulation. *J Control Release* 2019; **309**: 11-24 [PMID: 31326462 DOI: 10.1016/j.jconrel.2019.07.022]
2 **Cruz FF**, Rocco PRM. The potential of mesenchymal stem cell therapy for chronic lung disease. *Expert Rev Respir Med* 2020; **14**: 31-39 [PMID: 31608724 DOI: 10.1080/17476348.2020.1679628]
3 **Muhammad SA**, Abbas AY, Saidu Y, Fakurazi S, Bilbis LS. Therapeutic efficacy of mesenchymal stromal cells and secretome in pulmonary arterial hypertension: A systematic review and meta-analysis. *Biochimie* 2020; **168**: 156-168 [PMID: 31678635 DOI: 10.1016/j.biochi.2019.10.016]
4 **Gomberg-Maitland M**, Bull TM, Saggar R, Barst RJ, Elgazayerly A, Fleming TR, Grimminger F, Rainisio M, Stewart DJ, Stockbridge N, Ventura C, Ghofrani AH, Rubin LJ. New trial designs and potential therapies for pulmonary artery hypertension. *J Am Coll Cardiol* 2013; **62**: D82-D91 [PMID: 24355645 DOI: 10.1016/j.jacc.2013.10.026]
5 **Hernanda PY**, Pedroza-Gonzalez A, van der Laan LJ, Bröker ME, Hoogduijn MJ, Ijzermans JN, Bruno MJ, Janssen HL, Peppelenbosch MP, Pan Q. Tumor promotion through the mesenchymal stem cell compartment in human hepatocellular carcinoma. *Carcinogenesis* 2013; **34**: 2330-2340 [PMID: 23740837 DOI: 10.1093/carcin/bgt210]
6 **Robey TE**, Saiget MK, Reinecke H, Murry CE. Systems approaches to preventing transplanted cell death in cardiac repair. *J Mol Cell Cardiol* 2008; **45**: 567-581 [PMID: 18466917 DOI: 10.1016/j.yjmcc.2008.03.009]
7 **Yu X**, Cohen DM, Chen CS. miR-125b Is an adhesion-regulated microRNA that protects mesenchymal stem cells from anoikis. *Stem Cells* 2012; **30**: 956-964 [PMID: 22331826 DOI: 10.1002/stem.1064]
8 **Lee S**, Choi E, Cha MJ, Hwang KC. Cell adhesion and long-term survival of transplanted mesenchymal stem cells: a prerequisite for cell therapy. *Oxid Med Cell Longev* 2015; **2015**: 632902 [PMID: 25722795 DOI: 10.1155/2015/632902]
9 **Paoli P**, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. *Biochim Biophys Acta* 2013; **1833**: 3481-3498 [PMID: 23830918 DOI: 10.1016/j.bbamcr.2013.06.026]
10 **Terasaki M**, Iida T, Kikuchi F, Tamura K, Endo T, Kuramitsu Y, Tanaka T, Maeda H, Miyashita K, Mutoh M. Fucoxanthin potentiates anoikis in colon mucosa and prevents carcinogenesis in AOM/DSS model mice. *J Nutr Biochem* 2019; **64**: 198-205 [PMID: 30530259 DOI: 10.1016/j.jnutbio.2018.10.007]
11 **Yang J**, Zheng Z, Yan X, Li X, Liu Z, Ma Z. Integration of autophagy and anoikis resistance in solid tumors. *Anat Rec (Hoboken)* 2013; **296**: 1501-1508 [PMID: 23963853 DOI: 10.1002/ar.22769]
12 **Zhang Q**, Cheng X, Zhang H, Zhang T, Wang Z, Zhang W, Yu W. Dissecting molecular mechanisms underlying H(2)O(2)-induced apoptosis of mouse bone marrow mesenchymal stem cell: role of Mst1 inhibition. *Stem Cell Res Ther* 2020; **11**: 526 [PMID: 33298178 DOI: 10.1186/s13287-020-02041-7]
13 **Avruch J**, Zhou D, Fitamant J, Bardeesy N, Mou F, Barrufet LR. Protein kinases of the Hippo pathway: regulation and substrates. *Semin Cell Dev Biol* 2012; **23**: 770-784 [PMID: 22898666 DOI: 10.1016/j.semcdb.2012.07.002]
14 **Yu W**, Chen H, Yang H, Ding J, Xia P, Mei X, Wang L, Chen S, Zou C, Wang LX. Dissecting Molecular Mechanisms Underlying Pulmonary Vascular Smooth Muscle Cell Dedifferentiation in Pulmonary Hypertension: Role of Mutated Caveolin-1 (Cav1(F92A))-Bone Marrow Mesenchymal Stem Cells. *Heart Lung Circ* 2019; **28**: 1587-1597 [PMID: 30262154 DOI: 10.1016/j.hlc.2018.08.002]
15 **Chou LY**, Chen CH, Chuang SC, Cheng TL, Lin YH, Chou HC, Fu YC, Wang YH, Wang CZ. Discoidin Domain Receptor 1 Regulates Runx2 during Osteogenesis of Osteoblasts and Promotes Bone Ossification *via* Phosphorylation of p38. *Int J Mol Sci* 2020; **21** [PMID: 33003599 DOI: 10.3390/ijms21197210]
16 **Song H**, Cha MJ, Song BW, Kim IK, Chang W, Lim S, Choi EJ, Ham O, Lee SY, Chung N, Jang Y, Hwang KC. Reactive oxygen species inhibit adhesion of mesenchymal stem cells implanted into ischemic myocardium *via* interference of focal adhesion complex. *Stem Cells* 2010; **28**: 555-563 [PMID: 20073042 DOI: 10.1002/stem.302]
17 **Debnath J**. Detachment-induced autophagy during anoikis and lumen formation in epithelial acini. *Autophagy* 2008; **4**: 351-353 [PMID: 18196957 DOI: 10.4161/auto.5523]
18 **Tang Q**, Chen Q, Lai X, Liu S, Chen Y, Zheng Z, Xie Q, Maldonado M, Cai Z, Qin S, Ho G, Ma L. Malignant transformation potentials of human umbilical cord mesenchymal stem cells both spontaneously and *via* 3-methycholanthrene induction. *PLoS One* 2013; **8**: e81844 [PMID: 24339974 DOI: 10.1371/journal.pone.0081844]
19 **Gilmore AP**. Anoikis. *Cell Death Differ* 2005; **12** Suppl 2: 1473-1477 [PMID: 16247493 DOI: 10.1038/sj.cdd.4401723]
20 **Tuloup-Minguez V**, Greffard A, Codogno P, Botti J. Regulation of autophagy by extracellular matrix glycoproteins in HeLa cells. *Autophagy* 2011; **7**: 27-39 [PMID: 20980830 DOI: 10.4161/auto.7.1.13851]
21 **Hynes RO**. The extracellular matrix: not just pretty fibrils. *Science* 2009; **326**: 1216-1219 [PMID: 19965464 DOI: 10.1126/science.1176009]
22 **Frisch SM**, Vuori K, Ruoslahti E, Chan-Hui PY. Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol* 1996; **134**: 793-799 [PMID: 8707856 DOI: 10.1083/jcb.134.3.793]
23 **Giancotti FG**, Ruoslahti E. Integrin signaling. *Science* 1999; **285**: 1028-1032 [PMID: 10446041 DOI: 10.1126/science.285.5430.1028]
24 **Huang H**, Du W, Brekken RA. Extracellular Matrix Induction of Intracellular Reactive Oxygen Species. *Antioxid Redox*

Signal 2017; **27**: 774-784 [PMID: 28791881 DOI: 10.1089/ars.2017.7305]

25 **Li K**, Zhao G, Ao J, Gong D, Zhang J, Chen Y, Li J, Huang L, Xiang R, Hu J, Lin P, Wei Y. ZNF32 induces anoikis resistance through maintaining redox homeostasis and activating Src/FAK signaling in hepatocellular carcinoma. *Cancer Lett* 2019; **442**: 271-278 [PMID: 30439540 DOI: 10.1016/j.canlet.2018.09.033]

26 **Mao Y**, Qiao JD, Chen S, Zhou X, Wang Z, Cai S, Li L, Luo Y. Kallistatin Inhibits Anoikis Resistance and Metastasis of Ectopic Endometrium Cells by Modulating MnSOD and Caspase 3 Signaling. *Reprod Sci* 2021; **28**: 1012-1019 [PMID: 33449348 DOI: 10.1007/s43032-020-00421-1]

27 **Li L**, Tan J, Miao Y, Lei P, Zhang Q. ROS and Autophagy: Interactions and Molecular Regulatory Mechanisms. *Cell Mol Neurobiol* 2015; **35**: 615-621 [PMID: 25722131 DOI: 10.1007/s10571-015-0166-x]

28 **Idelchik MDPS**, Begley U, Begley TJ, Melendez JA. Mitochondrial ROS control of cancer. *Semin Cancer Biol* 2017; **47**: 57-66 [PMID: 28445781 DOI: 10.1016/j.semcancer.2017.04.005]

29 **Wang S**, Zhou L, Ling L, Meng X, Chu F, Zhang S, Zhou F. The Crosstalk Between Hippo-YAP Pathway and Innate Immunity. *Front Immunol* 2020; **11**: 323 [PMID: 32174922 DOI: 10.3389/fimmu.2020.00323]

30 **Galland S**, Stamenkovic I. Mesenchymal stromal cells in cancer: a review of their immunomodulatory functions and dual effects on tumor progression. *J Pathol* 2020; **250**: 555-572 [PMID: 31608444 DOI: 10.1002/path.5357]

31 **Timaner M**, Tsai KK, Shaked Y. The multifaceted role of mesenchymal stem cells in cancer. *Semin Cancer Biol* 2020; **60**: 225-237 [PMID: 31212021 DOI: 10.1016/j.semcancer.2019.06.003]

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