



REVIEW ARTICLE

Emerging roles of circular RNAs in stem cells

Mengru Wang, Juan Wu, Pan Wu, Yuhong Li*

Department of Cell Biology, Army Medical University, Chongqing 400038, China

Received 9 March 2022; received in revised form 5 May 2022; accepted 14 May 2022
Available online 31 May 2022

KEYWORDS

Circular RNA;
Differentiation;
Proliferation;
Stem cell;
Stem cell-derived
exosomes

Abstract Circular RNAs (circRNAs) are a novel class of noncoding RNAs that widely exist in eukaryotes. As a new focus in the field of molecular regulation, circRNAs have attracted much attention in recent years. Previous studies have confirmed that circRNAs are associated with many physiological and pathological processes. CircRNAs also participate in the regulation of stem cells. Stem cells have the properties of self-renewal and differentiation, which make stem cell therapy popular. CircRNAs may serve as new targets in stem cell therapy due to their regulation in stem cells. However, the underlying relationships between circRNAs and stem cells are still being explored. In this review, we briefly summarize the effects of circRNAs on stem cells, in the context of biological activities, aging and apoptosis, and aberrant changes. Moreover, we also examine the biological roles of stem cell-derived exosomal circRNAs. We believe our review will provide insights into the effects of circRNAs on stem cells.

© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

Circular RNAs (circRNAs), which have a covalently closed-loop structure, are a novel class of RNAs that are abundant in eukaryocytes. CircRNAs are circular products of precursor mRNA (pre-mRNA) formed by back-splicing, in which a downstream 5' donor site is joined to an upstream 3' acceptor site.¹ This process mainly leads to the formation of exonic circRNAs or uncommonly forms exon–intron

circRNAs.² The appearance of intronic circRNAs benefits from the lariat intermediate structure.³ In addition, transcription termination failure and chromosomal translocations also contribute to the biogenesis of circRNAs.^{4,5} CircRNA biogenesis is coupled with pre-mRNA transcription by Pol II and competes with its linear counterparts. Biogenesis can also be regulated by core spliceosomal machinery, cis elements, RNA-binding proteins (RBPs), and circRNA turnover.⁶ Although circRNAs were first discovered in 1976,⁷ their underlying role was not truly understood until 2013, when circRNAs were found to play a role by sponging miRNAs.^{8,9} To date, researchers revealed that circRNAs could be translated into proteins, regulate transcription and interact with proteins besides sponging miRNAs.¹⁰ Due to advances in RNA sequencing technology, we found that circRNAs were closely related to many

* Corresponding author. Gaotanyan Street No.30, Shapingba District, Chongqing 400038, China.

E-mail address: liyuhongtmmu@hotmail.com (Y. Li).

Peer review under responsibility of Chongqing Medical University.

physiological and pathological conditions.⁶ CircRNAs also play essential roles in the regulation of disease processes such as cancer, cardiovascular disease, and renal disease,^{11–13} and these unprecedented discoveries may change current therapeutic strategies in the future.

It is well known that stem cells have self-renewal and multidirectional differentiation potential, play critical roles in growth and development in humans. Furthermore, stem cell dysfunction is highly associated with many diseases. For example, hematopoietic stem cell (HSC) dysfunction can directly lead to aplastic anemia (AA). Thus, an increasing number of people are turning their attention to stem cell therapy. In cell therapy, stem cells can be used to replace damaged cells or regenerate organs, and in drug development, disease-specific cell lines can also be used.¹⁴ At present, HSC transplantation (HSCT) has been widely used to treat a variety of hematological diseases.¹⁵ Stem cell therapy has brought new hope for treating certain diseases.

However, to utilize the potential abilities of stem cells for therapy, we must determine the mechanisms associated with their growth and development. CircRNAs, which are a new focus in the field of molecular regulation, have attracted much attention for their effects on stem cells. In recent years, many researchers have reported the important roles of circRNAs in regulating stem cell fate.¹⁶ CircRNAs usually act as miRNA sponges to regulate stem cell differentiation.¹⁷ For example, circFOXP1 could promote osteogenic differentiation in adipose-derived mesenchymal stem cells (ADSCs) by sponging miR-33a-5p.¹⁸ In addition, circRNAs can regulate transcription and translation,¹⁰ but these functions have not been clear in stem cells until now. CircRNAs can affect stem cells in many ways, including in the context of biological activities, aging and apoptosis, and aberrant changes. Moreover, stem cell-derived exosomes that contain circRNAs can also have vital effects on organs or tissues. Human umbilical cord mesenchymal stem cell-derived exosomes (hucMSC-Exos) prevent pyroptosis and repair ischemic muscle injury by releasing circHIPK3, which can enhance the expression of FOXO3a.¹⁹ These results suggest that stem cell therapy can target circRNAs. However, the distinct relationships between circRNAs and stem cells are not clear.

In this review, we introduce the changes induced by circRNAs in stem cells (Fig. 1), including effects on biological activities, aging and apoptosis, aberrant changes and the effects of stem cell-derived exosomal circRNAs.

CircRNAs regulate the biological activities of stem cells

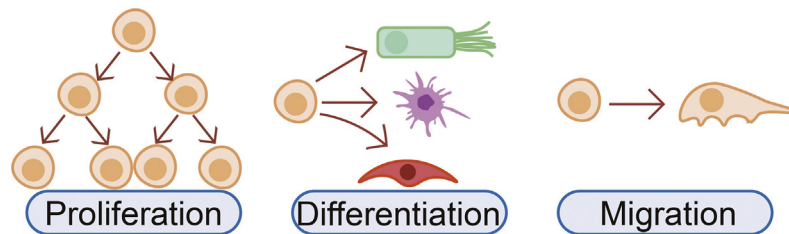
The effects of circRNAs on stem cell proliferation

The high proliferation capacity of stem cells is critical to the maintenance of tissue and organ homeostasis. The proliferation of stem cells is necessary for tissue or organ repair and self-renewal. In recent years, many researchers have found that circRNAs can promote stem cell proliferation. In human periodontal ligament stem cells (PDLSCs), the circRNA CDR1as was shown to promote proliferation by sponging miR-7 to activate the ERK signaling pathway in a

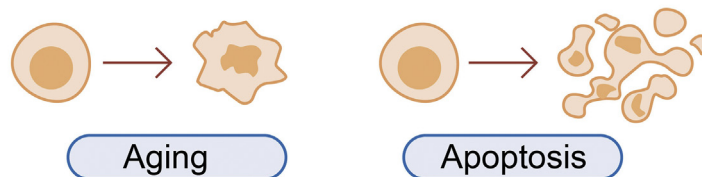
lipopolysaccharide-induced inflammatory microenvironment.²⁰ In addition, circMAP3K11 could also facilitate the proliferation of PDLSCs in an inflammatory microenvironment by sponging miR-511.²¹ The functions of CDR1as and circMAP3K11 in PDLSCs may promote periodontal tissue regeneration and reduce the injury caused by periodontitis. In mesenchymal stem cells (MSCs), many studies have verified the important role of circRNAs in determining their fate.²² In fact, MSCs perform good therapeutic effects on many diseases like bone and cartilage, neurological, cardiovascular, and autoimmune diseases.²³ In regard to promoting the proliferation of MSCs, hsa_circ_0066523 promotes the proliferation of bone marrow-derived mesenchymal stem cells (BMSCs) by repressing phosphatase and tensin homolog (PTEN), thereby activating the AKT pathway.²⁴ Mmu_circRNA_003795 can promote BMSC proliferation through calcitonin gene-related peptide (CGRP) by increasing FOS like 2 AP1 transcription factor subunit (FOSL2) expression by sponging miR-504-3p.²⁵ CircRNA_0001052 accelerates BMSC proliferation by sponging miR-124-3p through the Wnt4/beta-catenin pathway in low-level laser irradiation treatment (LLLI).²⁶ For clinical application, LLLI is a potential method to stimulate BMSC proliferation in patients. Circ_FBLN1, circ-DAB1 and circPVT1 also play positive roles in BMSC proliferation and have essential effects on BMSC differentiation or apoptosis at the same time.^{27–29} CDR1as was confirmed to take a part in maintaining proliferation and differentiation of hucMSCs.³⁰ In myoblasts, circSVIL could facilitate bovine myoblast proliferation by inhibiting STAT1 phosphorylation.³¹ In another study, circSVIL functioned as a miR-203 sponge and upregulated the mRNA levels of c-JUN and MEF2C to promote the proliferation of myoblasts in chickens.³² CircHIPK3 could act as a sponge of miR-7 to promote murine myoblast proliferation and function as a sponge of miR-30a-3p to promote chicken myoblast proliferation.^{33,34} Circ-ZNF609 was also found to have a positive effect on myoblast proliferation; surprisingly, researchers found that circ-ZNF609 could be translated into protein because it contains an open reading frame.³⁵ In addition, circHUWE1, circTTN, circINSR, circRILPL1, circCPE and circFGFR2 can also promote myoblast proliferation by sponging corresponding microRNAs.^{36–41} In MC3T3-E1 cells, the circRNA AFF4 acts as a sponge of miR-7223-5p, thereby promoting MC3T3-E1 cell proliferation and inhibiting apoptosis.⁴²

CircRNAs can also inhibit the cell proliferation. For instance, circ-Smad5 can restrain the proliferation of JB6 cells by reducing the activation of wnt/lef/cyclind1 signaling.⁴³ Regarding stem cell proliferation, circTTC3 inhibits the proliferation of neural stem cells (NSCs) through the miR-372-3p/Toll-like receptor 4 (TLR4) axis, and the depletion of circTTC3 promotes proliferation and upregulates nestin and β -tubulin III expression in NSCs. Moreover, the depletion of circTTC3 reduces cerebral infarction, neurological score, and brain water content in mouse model, which implies that circTTC3 can be a target in treating cerebral ischemia/reperfusion injury during stroke.⁴⁴ CircRNA_25487 inhibits BMSC proliferation by sequestering miR-134-3p, which can inhibit bone repair in trauma-induced osteonecrosis of the femoral head.⁴⁵ In myoblasts, circMYL1, circSNX29 and circFUT10 can reduce

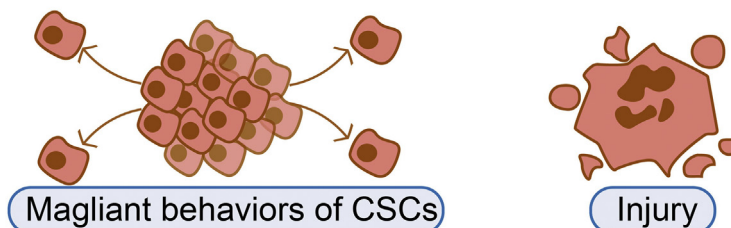
A. CircRNAs regulate the biological activities of stem cells



B. CircRNAs regulate the aging and apoptosis of stem cells



C. CircRNAs regulate the aberrant changes of stem cells



D. Stem cell-derived exosomal circRNAs and their functions

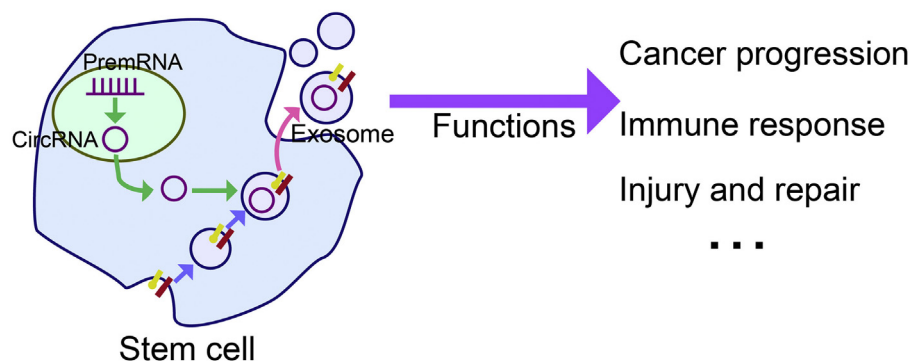


Figure 1 Effects of circRNAs on stem cells. Abbreviations: circRNA, circular RNA; CSC, cancer stem cell; premRNA, precursor mRNA.

myoblast proliferation by sponging miR-2400, miR-744 and miR-133a, respectively.^{46–48} The detailed studies were summarized in Table 1.

The effects of circRNAs on stem cell differentiation

It is universally acknowledged that stem cell differentiation is indispensable for human development. Stem cell-directed differentiation enables the specific functions of organs and tissues. Mechanistically, cell differentiation involves the selective expression of genes in time and space. At the molecular level, the role of circRNAs in stem cell differentiation has been a research hotspot for several

years. Here, we summarize previous research on the role of circRNAs in the differentiation of various types of stem cells (Fig. 2).

The effects of circRNAs on mesenchymal stem cell (MSC) differentiation

MSCs are pluripotent stem cells that have the potential to differentiate into a variety of tissue cells and have strong immunomodulatory effects. In stem cell therapy, MSCs are one of the most widely used and safest stem cells at present. Moreover, MSCs can be derived from various tissues, such as bone marrow, adipose tissue, umbilical cord and

Table 1 Roles of circRNAs in stem cell proliferation.

CircRNAs	Species	Cell types	Mechanisms	Target genes or pathways	Effects on proliferation	Ref
CDR1as	Homo sapiens	PDLSC	Sponge miR-7	ERK signaling pathway	Promote	20
CircMAP3K11	Homo sapiens	PDLSC	Sponge miR-511	TLR4	Promote	21
Hsa_circ_0066523	Homo sapiens	BMSC	Repress PTEN	AKT signaling pathway	Promote	24
Mmu_circRNA_003795	Mus	BMSC	Sponge miR-504-3p	FOSL2	Promote	25
CircRNA_0001052	Mus	BMSC	Sponge miR-124-3p	Wnt4/beta-catenin pathway	Promote	26
Circ_FBLN1	Homo sapiens	BMSC	Sponge let-7i-5p	FZD4, Wnt/beta-catenin pathway	Promote	27
Circ-DAB1	Homo sapiens	BMSC	Sponge miR-1270 and miR-944	NOTCH/RBPJ pathway	Promote	28
CircPVT1	Rattus	BMSC	Sponge miR-21-5p	Smad7/TGFbeta pathway	Promote	29
CDR1as	Homo sapiens	hucMSC	Unknown	STFs	Promote	30
CircSVIL	Bos taurus	Myoblast	Inhibit STAT1 phosphorylation	Downstream signal cascade of STAT1	Promote	31
CircHIPK3	Gallus gallus	Myoblast	Sponge miR-203	MEF2C and c-JUN	Promote	32
	Mus		Sponge miR-7	TCF12	Promote	33
	Gallus gallus		Sponge miR-30a-3p	Unknown	Promote	34
Circ-ZNF609	Mus and Homo sapiens	Myoblast	Unknown	Unknown	Promote	35
CircHUWE1	Bos taurus	Myoblast	Sponge miR-29b	AKT signaling pathway	Promote	36
CircTTN	Bos taurus	Myoblast	Sponge miR-432	IGF2/PI3K/AKT pathway	Promote	37
CircINSR	Bos taurus	Myoblast	Sponge miR-34a	Bcl-2 and CyclinE2	Promote	38
CircRILPL1	Bos taurus	Myoblast	Sponge miR-145	IGF1R/PI3K/AKT pathway	Promote	39
CircCPE	Bos taurus	Myoblast	Sponge miR-138	FOXC1	Promote	40
CircFGFR2	Gallus gallus	Myoblast	Sponge miR-133a-5p and miR-29b-1-5p	Unknown	Promote	41
CircRNA AFF4	Mus	MC3T3-E1 cell	Sponge miR-7223-5p	PIK3R1	Promote	42
Circ-Smad5	Mus	JB6 cell	Unknown	Wnt/lef/cyclind1 signaling	Inhibit	43
CircTTC3	Mus	NSC	Sponge miR-372-3p	TLR4	Inhibit	44
CircRNA_25487	Homo sapiens	BMSC	Sponge miR-134-3p	p21	Inhibit	45
CircMYL1	Bos taurus	Myoblast	Sponge miR-2400	Unknown	Inhibit	46
CircSNX29	Bos taurus	Myoblast	Sponge miR-744	Wnt5a/Ca (2+) signaling pathway	Inhibit	47
CircFUT10	Bos taurus	Myoblast	Sponge miR-133a	Unknown	Inhibit	48
CircFAM188B	Gallus gallus	SMSC	Encode circFAM188B-103aa	Unknown	Promote	94
CircFNDC3AL	Gallus gallus	SMSC	Sponge miR-204	BCL9	Promote	95
CircPPP1R13B	Gallus gallus	SMSC	Sponge miR-9-5p	IGF/PI3K/AKT signaling pathway	Promote	96
CircITSN2	Gallus gallus	Myoblast	Sponge miR-218-5p	LMO7	Promote	100
CircEch1	Bos taurus	Myoblast	Unknown	Unknown	Inhibit	101
CircLMO7	Bos taurus	Myoblast	Sponge miR-378a-3p	Unknown	Promote	102

BMSC, bone marrow-derived mesenchymal stem cell; c-JUN, c-Jun N-terminal kinase; FOSL2, FOS like 2 AP-1 transcription factor sub-unit; FZD4, frizzled class receptor 4; hucMSC, mesenchymal stem cell derived from human umbilical cord; IGF1R, insulin-like growth factor 1 receptor; IGF2, insulin-like growth factor 2; MC3T3-E1 cell, mouse embryo osteoblast precursor cell; MEF2C, myocyte enhancer factor 2 C; NSC, neural stem cell; PDLSC, periodontal ligament stem cell; PI3K, phosphatidylinositol 3-kinase; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PTEN, phosphatase and tensin homolog; RBPJ, recombination signal-binding protein for immunoglobulin kappa J region; SMSC, skeletal muscle satellite cell; STAT1, signal transducing activator of transcription 1; STFs, stemness transcription factors; TCF12, transcription factor 12; TGF, transforming growth factor; TLR4, toll-like receptor 4.

placental chorionic plates. Here, we introduce the function of circRNAs in the differentiation of MSCs derived from different tissues.

In BMSCs, circRNAs modulate osteogenic differentiation. The dysfunction of BMSC osteogenic differentiation is associated with a range of diseases including osteoporosis,

osteoarthritis and osteonecrosis. Microarray analysis revealed changes in circRNAs in BMSCs during osteoblast differentiation and showed that 3938 circRNAs were up-regulated and 1505 were downregulated in BMSCs at day 7 (D7) compared with day 0 (D0). The study validated the expression of the hub miRNA, miR-199b-5p, which was

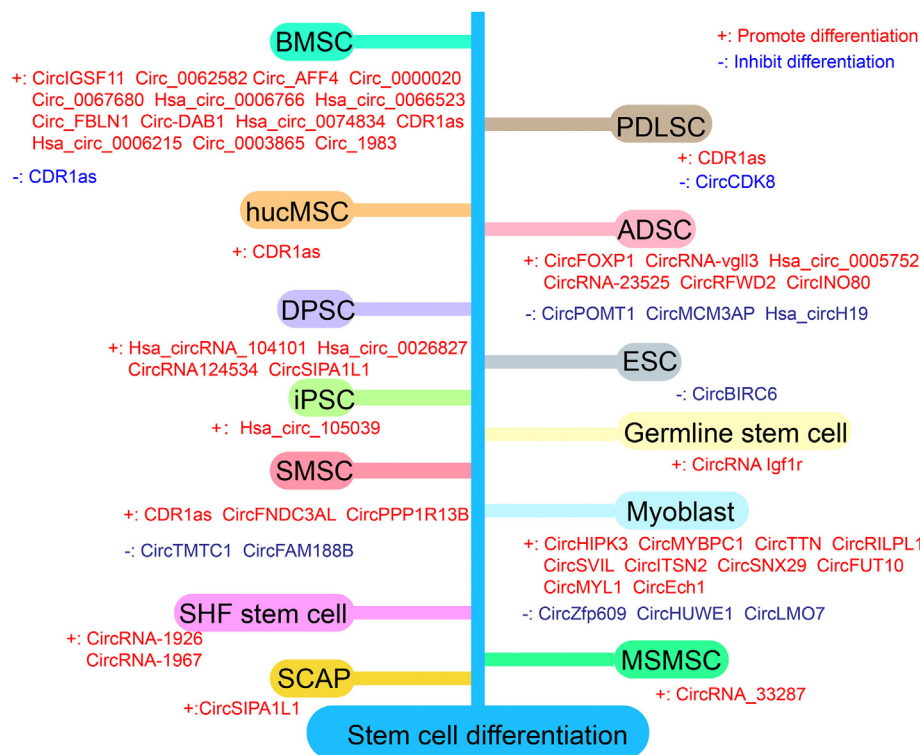


Figure 2 CircRNAs in stem cell differentiation. The figure shows that the role of circRNAs in the differentiation of different stem cells. CircRNAs that can promote differentiation were marked in red and circRNAs that can inhibit differentiation were marked in blue. CDR1as can promote adipogenic differentiation while suppresses osteogenic differentiation in BMSCs. Abbreviations: ADSC, adipose-derived mesenchymal stem cell; BMSC, bone marrow-derived mesenchymal stem cell; DPSC, dental pulp stem cell; ESC, embryonic stem cell; HSC, hematopoietic stem cell; hucMSC, human umbilical cord mesenchymal stem cell; iPSC, induced pluripotent stem cell; MSMSC, maxillary sinus membrane stem cell; PDLSC, periodontal ligament stem cell; SCAP, stem cells from apical papilla; SHF, secondary hair follicle; SMSC, skeletal muscle satellite cell.

associated with circIGSF11. Silencing circIGSF11 facilitated osteoblast differentiation and increased the expression of miR-199b-5p.⁴⁹ In fact, circRNAs typically act as sponges for miRNAs and reduce their effects on stem cell differentiation.¹⁷ In addition, circRNAs often have specific expression patterns. Circ_0062582 overexpression significantly promotes osteogenic differentiation by upregulating core-binding factor subunit beta (CBFB) expression by targeting miR-145.⁵⁰ CBFB plays an important role in stabilizing RUNX family proteins, which are essential for osteogenic differentiation.⁵¹ By sponging miR-135a-5p, circ_AFF4 can trigger high expression of FNDC5/Irisin, which activates the SMAD1/5 pathway to promote osteogenic differentiation.⁵² Circ_0000020 promotes osteogenic differentiation by sponging miR-142-5p, which upregulates the bone morphogenetic protein BMP2.⁵³ Similarly, circ_0067680 and hsa_circ_0006766 also accelerate osteogenic differentiation in BMSCs through the circ_0067680/miR-4429/CTNNB1/Wnt/ β -catenin pathway and hsa_circ_0006766/miR-4739/Notch2 regulatory axis, respectively.^{54,55} In addition to promoting osteogenic differentiation, some circRNAs also have other effects on BMSCs. For example, hsa_circ_0066523 facilitates osteogenic differentiation and proliferation in BMSCs by suppressing PTEN expression.²⁴ Circ_FBLN1 and circ-DAB1 can also promote proliferation and osteogenic differentiation

through their specific expression patterns.^{27,28} In BMSCs from patients with nonunion, hsa_circ_0074834 is found to be downregulated by high-throughput assay. As a ceRNA for miR-942-5p, hsa_circ_0074834 not only accelerates osteogenic differentiation in BMSCs but also promotes the osteogenesis-angiogenesis coupling process by increasing ZEB1 and VEGF expression.⁵⁶ Analogously, hsa_circ_0006215, which decreases in BMSCs from patients with osteoporosis, expedites osteogenic differentiation in BMSCs and strengthens osteogenesis-angiogenesis coupling by competitively binding to miR-942-5p and regulating RUNX2 and VEGF expression. Hsa_circ_0006215 also promotes bone regeneration in a cortical bone defect model.⁵⁷ In addition, changes in the extracellular environment of BMSCs can also cause changes in circRNAs, which may affect differentiation. Melatonin can suppress circ_0003865 expression and increase miR-3653-3p expression, and miR-3653-3p inhibits the expression of growth arrest-specific gene 1 (GAS1) to promote osteogenic differentiation. Moreover, circ_0003865 silencing repressed osteoporosis development in mouse model.⁵⁸ In dicalcium silicate microparticle (C2S)-treated BMSCs, circ_1983 expression was elevated, and circ_1983 can sponge miR-6931 to facilitate osteogenic differentiation by increasing GAS7 and RUNX2 expression.⁵⁹ Surface mechanical attrition treatment (SMAT) with titanium can also alter osteogenic

differentiation by altering the expression of circRNAs. Bioinformatics analysis using miRWalk3.0 software showed that 51 circRNAs were predicted to construct ceRNA networks with osteogenesis-related mRNAs and miRNAs.⁶⁰ It is worth noting that circRNAs can affect the adipogenic differentiation of BMSCs as well. CircRNA CDR1as promotes adipogenesis and suppresses osteogenesis in BMSCs in steroid-induced osteonecrosis of the femoral head through the CDR1as/miR-7-5p/WNT5B axis.⁶¹ The axis provides a novel target for treating osteonecrosis.

In ADSCs, circRNAs also influence osteogenic differentiation. A circRNA microarray showed that a total of 171 circRNAs were upregulated and 119 were downregulated during induced osteogenic differentiation in ADSCs compared with noninduced cells.⁶² This result hints at the importance of circRNAs in the osteogenic differentiation of ADSCs. CircFOXP1 acts as a miR-33a-5p sponge to upregulate the expression of FOXP1, which controls several differentiation pathways, thereby promoting osteogenic differentiation in ADSCs.¹⁸ CircRNA-vgll3 facilitates ADSC osteogenic differentiation by activating the integrin $\alpha 5$ (Itga5) pathway by sequestering miR-326-5p. In a rat bone defects model, circRNA-vgll3-modified ADSCs obviously enhance new bone formation.⁶³ This confirms the value of circRNA-vgll3 in healing injured bone. Hsa_circ_0005752 can sponge miR-496 to mediate ADSC osteogenic differentiation, and RUNX3 can enhance the level of hsa_circ_0005752.⁶⁴ CircRNA-23525 regulates RUNX2 expression by targeting miR-30a-3p and promotes osteogenic differentiation in ADSCs.⁶⁵ Similarly, circRFWD2 and circINO80 can interact with hsa-miR-6817-5p in ADSCs and play a positive role in recombinant NELL-1-induced osteogenesis.⁶⁶ Conversely, circPOMT1 and circMCM3AP inhibit the osteogenic differentiation of ADSCs by targeting miR-6881-3p.⁶⁷ In the context of ADSC adipogenic differentiation, knockdown of hsa_circH19 promotes this process because hsa_circH19 restrains the expression of polypyrimidine tract-binding protein 1 (PTBP1). In fact, hsa_circH19 is upregulated in patients with metabolic syndrome and correlates with lipid-related parameters.⁶⁸ The suppression of adipogenic differentiation of ADSC is one of the main causes of disorders associated with metabolic syndrome.⁶⁹ Targeting hsa_circH19 may improve symptoms of metabolic syndrome.

In hucMSCs, CDR1as may play an important role during adipogenesis and osteogenesis. After CDR1as knockdown, the expression of stemness transcription factors (STFs) was downregulated, and the adipogenesis and osteogenesis potential of hucMSCs was impaired.³⁰ During the differentiation of hucMSCs into cardiomyocyte-like cells induced by 5-aza, RT-PCR showed that a total of 226 circRNAs were differentially expressed, and gene ontology (GO) analysis identified a number of cell proliferation- and differentiation-related physiological processes and pathways.⁷⁰

The effects of circRNAs on PDLSC differentiation

PDLSCs are an important focus in periodontal regeneration and alveolar bone repair. During PDLSC osteogenic differentiation, an analysis revealed that 147 lncRNAs and 1382 circRNAs were predicted to bind with 148 common miRNAs

and compete for miRNA binding sites with 744 messenger RNAs, and these miRNAs had been predicted to regulate the pluripotency of stem cells.⁷¹ Another analysis showed that the expression of circRNAs during PDLSC osteogenic differentiation was stage-specific. The expression profiles of circRNAs are altered during different stages of osteogenic differentiation.⁷² Thus, the roles of circRNAs are worthy of intensive study. CDR1as was markedly upregulated, while miR-7 was significantly downregulated during PDLSC osteoblastic differentiation.⁷³ The study also showed that the 3' untranslated region (UTR) of growth and differentiation factor-5 (GDF5) contained miR-7 binding sites. GDF5 is closely associated with osteogenic differentiation.⁷⁴ Therefore, CDR1as acts upstream of miR-7 and inhibits the effect of miR-7 by targeting GDF5, which enhances Smad1/5/8 and p38 MAPK phosphorylation and promotes osteoblastic differentiation.⁷³ In addition, by sponging miR-7, CDR1as can increase the expression of the stemness-related gene KLF4 to promote PDLSC multidifferentiation and migration.⁷⁵ In cobalt chloride (CoCl₂) induced hypoxia, circCDK8 can induce autophagy and apoptosis through mTOR signaling and then suppress the osteogenic differentiation in PDLSCs.⁷⁶ Hypoxia of periodontal tissues is usually an outcome of periodontitis. Therefore, circCDK8 could be a therapeutic target of periodontitis accompanied by hypoxia.

The effects of circRNAs on dental pulp stem cell (DPSC) differentiation

DPSCs are fibroblasts isolated from dental pulp tissue and are a kind of MSC. DPSCs are not an ideal candidate stem cells for prosthodontics but also play important roles in the repair of other tissues due to their excellent capacity for multidifferentiation. During the odontogenic differentiation of hDPSCs, a study identified 43 upregulated and 144 downregulated circRNAs by using qRT-PCR and microarrays. Among these, hsa_circRNA_104101 was shown to promote the odontogenic differentiation of hDPSCs.⁷⁷ In DPSC osteogenic differentiation, hsa_circ_0026827 can act a positive role by sponging miR-188-3p, which can decrease the Beclin-1-mediated autophagy and increase the expression of RUNX1.⁷⁸ CircRNA124534 can also promote DPSC osteogenic differentiation by activating the β -catenin pathway by binding to miR-496.⁷⁹ Moreover, circSIPA1L1 promotes osteogenesis in DPSCs by sponging miR-617 and further targeting Smad3, which is beneficial for osteogenic differentiation.⁸⁰ Hsa_circ_0026827, circRNA124534, and circSIPA1L1 could be a candidate target in DPSCs for treating diseases associated with bone formation.

The effects of circRNAs on embryonic stem cell (ESC) differentiation

ESCs are highly undifferentiated cells. ESCs can theoretically differentiate into all tissue and organ types. An analysis of hESC differentiation showed that the dominant forms of the lncRNAs RMST and FIRRE are circular, suggesting that the roles of circRNAs in hESCs cannot be neglected.⁸¹ CircBIRC6 can suppress hESC differentiation by directly interacting with miR-34a and miR-145 because miR-

34a/miR-145 modulate target genes that maintain pluripotency.⁸² In addition, the RBP FUS participates in circRNA expression in murine ESCs and plays a role in ESC-derived motor neuron differentiation. In this study, Lorenzo Erichelli et al used RNA sequencing analysis and showed that 3894 circRNAs among 2097 known genes were expressed in ESC-derived motor neurons.⁸³

The effects of circRNAs on hematopoietic stem cell (HSC) differentiation

HSCs can differentiate into a variety of myeloid cells and lymphocytes and have strong self-renewal ability. HSCT has become an important therapeutic method and has been used to treat many serious diseases. However, the role of circRNAs in HSCs remains unclear. Xia et al showed that the circRNA cia-cGAS can maintain homeostasis in the context of cGAS-mediated HSC exhaustion. Cia-cGAS has a strong affinity for cGAS and inhibits cGAS-mediated production of type I IFNs in long-term HSCs.⁸⁴ An analysis of circRNA expression in HSCs revealed that circRNAs were widely expressed. As hematopoiesis continues, the expression of circRNAs is altered. Moreover, circRNA expression is cell type specific during hematopoietic differentiation. High expression levels of circRNAs are observed in red blood cells (RBCs) and platelets. Intriguingly, circRNA expression is reduced in aging RBCs.⁸⁵ CircRNA may play an important role in the function of hemocyte. However, the underlying mechanism and function remain to be explored.

The effects of circRNAs on induced pluripotent stem cell (iPSC) differentiation

iPSCs have a strong capacity to differentiate into cells of all three germ layers, similar to ESCs, but iPSCs are easily obtained from somatic cells. Thus, the therapeutic application of iPSCs has become a hotspot but has faced a number of challenges.⁸⁶ In regard to the effects of circRNAs on iPSCs, a circRNA map of iPSCs of fetal origin showed the differences between human iPSCs from cord blood multipotent stromal cells and human iPSCs from adult skin fibroblasts. The study showed that the 9 out of 10 top circRNAs were predicted to interact with 27 miRNAs in hiPSCs from cord blood multipotent stromal cells, but their functions are still unknown.⁸⁷ In hiPSC-derived cardiomyocytes, a study showed more enriched expression of circRNAs than in undifferentiated hiPSCs. Moreover, circSLC8A1, circCACNA1D, circSPHKAP and circALPK2 may be biomarkers of cardiomyocytes due to their specificity.⁸⁸ CircSLC8A1 abnormally upregulated in heart tissues from patients with dilated cardiomyopathy. Furthermore, in hiPSC-derived cardiomyocytes, hsa_circ_105039 can sponge miR-17 and upregulate cyclinD2 expression to promote this process.⁸⁹

The effects of circRNAs on germline stem cell differentiation

High-throughput sequencing and RT-PCR revealed 18573 novel lncRNAs and 18822 circRNAs in murine germline stem cells. In addition, 8115 mRNAs, 3996 lncRNAs and 921

circRNAs exhibited sex-biased expression, which may be associated with sex-specific properties. The study also showed that lncRNA Meg3 and circRNA Igf1r can act as sponges of miRNA-15a-5p and increase the expression of the target genes *Inha*, *Acs13*, *Kif21b*, and *Igfbp2*.⁹⁰ These genes may participate in many biological processes. CircGFRalpha1 is an N6-methyladenosine (m6A)-modified circRNA that is stage-specifically expressed during female germline stem cells development. CircGFRalpha1 sponges miR-449 to enhance GFRalpha1 expression and activate the GDNF signaling pathway, thus facilitating female germline stem cell self-renewal. Moreover, the m6A writer METTL14 can promote the cytoplasmic export of m6A-modified circGFRalpha1.⁹¹

The effects of circRNAs on skeletal muscle satellite cell (SMSC) differentiation

SMSCs are the precursor cells of myoblasts and are a specific kind of adult stem cell in skeletal muscle. SMSCs are usually in a static state and are only activated when skeletal muscle is injured. CDR1as, which has attracted much attention, also plays a role in SMSC differentiation. CDR1as can bind to miR-7 to increase the expression of insulin-like growth factor-1 receptor (IGF1R) and promote SMSC differentiation in goat fetuses.⁹² CircTMTC1 can inhibit chicken SMSC differentiation by sponging miR-128-3p.⁹³ CircFAM188B can repress SMSC differentiation and induce SMSC proliferation. Surprisingly, circFAM188B encodes a protein called circFAM188B-103aa because circFAM188B contains an open reading frame. The function of circFAM188B-103aa is consistent with that of circFAM188B.⁹⁴ CircFNDC3AL and circPPP1R13B can facilitate SMSC proliferation and differentiation by targeting miR-204 and miR-9-5p, respectively.^{95,96} Therefore, circRNA has potential clinical application value in promoting myogenesis and repair of muscle injury.

The effects of circRNAs on myoblast differentiation

Myoblasts are essential for skeletal muscle development and regeneration. Myoblasts are easy to identify, culture and reproduce, and the studies of circRNAs in myoblasts are notably. It has been mentioned previously that circRNAs can impact myoblast proliferation; in fact, circRNAs also affect myoblast differentiation. CircRNAs can sponge miRNAs in myoblast differentiation as well. Circ-HIPK3 sponges miR-124 and miR-379 to promote the differentiation of C2C12 myoblasts.⁹⁷ CircMYBPC1 can directly bind to miR-23a to enhance the expression of myosin heavy chain (MyHC) and promote bovine myoblast differentiation.⁹⁸ In contrast, by sponging miR-194-5p, circZfp609 can increase the expression of BCL2 associated transcription factor 1 (BCLAF1) to inhibit C2C12 myoblast differentiation.⁹⁹ However, in many times, circRNAs can simultaneously regulate myoblast proliferation and differentiation. One case is that circRNAs synchronously facilitate myoblast proliferation and differentiation. These circRNAs include circTTN, circRILPL1, circSVIL, circHIPK3, circFGFR2, and circITSN2, which all play positive roles by sponging miRNAs.^{32–34,37,39,41,100} CircRNAs can also asynchronously regulate myoblast

Table 2 Roles of circRNAs in stem cell differentiation.

CircRNAs	Species	Cell types	Mechanisms	Target genes or pathways	Effects on differentiation	Ref
CircIGSF11	Homo sapiens	BMSC	Sponge miR-199b-5p	Unknown	Promote osteogenic differentiation	49
Circ_0062582	Homo sapiens	BMSC	Sponge miR-145	CBFB	Promote osteogenic differentiation	50
Circ_AFF4	Homo sapiens	BMSC	Sponge miR-135a-5p	SMAD1/5 pathway	Promote osteogenic differentiation	52
Circ_0000020	Rattus	BMSC	Sponge miR-142-5p	BMP2	Promote osteogenic differentiation	53
Circ_0067680	Homo sapiens	BMSC	Sponge miR-4429	CTNNB1/Wnt/beta-catenin pathway	Promote osteogenic differentiation	54
Hsa_circ_0006766	Homo sapiens	BMSC	Sponge miR-4739	Notch2	Promote osteogenic differentiation	55
Hsa_circ_0066523	Homo sapiens	BMSC	Repress PTEN	AKT signaling pathway	Promote osteogenic differentiation	24
Circ_FBLN1	Homo sapiens	BMSC	Sponge let-7i-5p	FZD4, Wnt/beta-catenin pathway	Promote osteogenic differentiation	27
Circ-DAB1	Homo sapiens	BMSC	Sponge miR-1270 and miR-944	NOTCH/RBPJ pathway	Promote osteogenic differentiation	28
Hsa_circ_0074834	Homo sapiens	BMSC	Sponge miR-942-5p	ZEB1, VEGF	Promote osteogenic differentiation and osteogenesis–angiogenesis coupling	56
Hsa_circ_0006215	Homo sapiens	BMSC	Sponge miR-942-5p	RUNX2, VEGF	Promote osteogenic differentiation and osteogenesis–angiogenesis coupling	57
Circ_0003865	Homo sapiens	BMSC	Sponge miR-3653-3p	GAS1	Promote osteogenic differentiation	58
Circ_1983	Mus	BMSC	Sponge miR-6931	GAS7 and RUNX2	Promote osteogenic differentiation	59
CDR1as	Homo sapiens	BMSC	Sponge miR-7-5p	WNT5B	Promote adipogenic differentiation Inhibit osteogenic differentiation	61
CircFOXP1	Homo sapiens	ADSC	Sponge miR-33a-5p	FOXP1	Promote osteogenic differentiation	18
CircRNA-vgll3	Rattus	ADSC	Sponge miR-326-5p	Itga5 pathway	Promote osteogenic differentiation	63
Hsa_circ_0005752	Homo sapiens	ADSC	Sponge miR-496	MDM2	Promote osteogenic differentiation	64
CircRNA-23525	Mus	ADSC	Sponge miR-30a-3p	RUNX2	Promote osteogenic differentiation	65
CircRFWD2	Homo sapiens	ADSC	Sponge	Unknown	Promote osteogenic differentiation	66
CircINO80	Homo sapiens	ADSC	hsa-miR-6817-5p	Unknown	Promote osteogenic differentiation	67
CircPOMT1	Homo sapiens	ADSC	Sponge miR-6881-3p	BMPs signaling pathway	Inhibit osteogenic differentiation	68
CircMCM3AP	Homo sapiens	ADSC	Bind to PTBP1	Unknown	Inhibit adipogenic differentiation	30
Hsa_circH19	Homo sapiens	ADSC	Bind to PTBP1	Unknown	Inhibit adipogenic differentiation	30
CDR1as	Homo sapiens	hucMSC	Unknown	STFs	Promote osteogenic differentiation and adipogenic differentiation	73
CDR1as	Homo sapiens	PDLSC	Sponge miR-7	GDF5	Promote osteogenic differentiation	75
CDR1as	Homo sapiens	PDLSC	Sponge miR-7	KLF4	Promote multidifferentiation	76
CircCDK8	Homo sapiens	PDLSC	Unknown	mTOR signaling, autophagy	Inhibit osteogenic differentiation	76

(continued on next page)

Table 2 (continued)

CircRNAs	Species	Cell types	Mechanisms	Target genes or pathways	Effects on differentiation	Ref
Hsa_circRNA_104101	Homo sapiens	DPSC	Unknown	Unknown	Promote odontogenic differentiation	77
Hsa_circ_0026827	Homo sapiens	DPSC	Sponge miR-188-3p	Beclin1, RUNX1	Promote osteogenic differentiation	78
CircRNA124534	Homo sapiens	DPSC	Sponge miR-496	β -catenin pathway	Promote osteogenic differentiation	79
CircSIPA1L1	Homo sapiens	DPSC	Sponge miR-617	Smad3	Promote osteogenic differentiation	80
CircBIRC6	Homo sapiens	ESC	Sponge miR-34a and miR-145	Pluripotency-associated genes	Inhibit differentiation	82
Cia-cGAS	Homo sapiens	HSC	Bind to cGAS	TypellFNs	Maintain the homeostasis in terms of cGAS-mediated HSC exhaustion	84
Hsa_circ_105039	Homo sapiens	iPSC	Sponge miR-17	CyclinD2	Promote cardiomyocyte differentiation	89
CircRNA Igf1r	Mus	Germline stem cell	Sponge miR-15a-5p,	Inha, Acsl3, Kif21b, Igfbp2	Promote germline stem cell differentiation, sex differentiation, reproductive process, etc.	90
CircGFRalpha1	Mus	Germline stem cell	Sponge miR-449	GFRalpha1, GDNF signaling pathway	Promote female germline stem cell self-renewal and express stage-specifically	91
CDR1as	<i>Capra hircus</i>	SMSC	Sponge miR-7	IGF1R	Promote SMSC differentiation	92
CircTMTTC1	Gallus gallus	SMSC	Sponge miR-128-3p	Unknown	Inhibit SMSC differentiation	93
CircFAM188B	Gallus gallus	SMSC	Encode circFAM188B-103aa	Unknown	Inhibit SMSC differentiation	94
CircFNDC3AL	Gallus gallus	SMSC	Sponge miR-204	BCL9	Promote SMSC differentiation	95
CircPPP1R13B	Gallus gallus	SMSC	Sponge miR-9-5p	IGF/PI3K/AKT pathway	Promote SMSC differentiation	96
Circ-HIPK3	Mus	Myoblast	Sponge miR-124 and miR-379	Unknown	Promote myoblast differentiation	97
CircMYBPC1	Bos taurus	Myoblast	Sponge miR-23a	MyHC	Promote myoblast differentiation	98
CircZfp609	Mus	Myoblast	Sponge miR-194-5p	BCLAF1	Inhibit myoblast differentiation	99
CircTTN	Bos taurus	Myoblast	Sponge miR-432	IGF2/PI3K/AKT Pathway	Promote myoblast differentiation	37
CircRILPL1	Bos taurus	Myoblast	Sponge miR-145	IGF1R/PI3K/AKT pathway	Promote myoblast differentiation	39
CircSVIL	Gallus gallus	Myoblast	Sponge miR-203	MEF2C and c-JUN	Promote myoblast differentiation	32
CircHIPK3	Mus	Myoblast	Sponge miR-7	TCF12	Promote myoblast differentiation	33
	Gallus gallus		Sponge miR-30a-3p	MEF2C	Promote myoblast differentiation	34
CircITSN2	Gallus gallus	Myoblast	Sponge miR-218-5p	LMO7	Promote myoblast differentiation	100
CircSNX29	Bos taurus	Myoblast	Sponge miR-744	Wnt5a/Ca (2+) signaling pathway	Promote myoblast differentiation	47
CircFUT10	Bos taurus	Myoblast	Sponge miR-133a	Unknown	Promote myoblast differentiation	48
CircMYL1	Bos taurus	Myoblast	Sponge miR-2400	Unknown	Promote myoblast differentiation	46
CircEch1	Bos taurus	Myoblast	Unknown	Unknown	Promote myoblast differentiation	100

Table 2 (continued)

CircRNAs	Species	Cell types	Mechanisms	Target genes or pathways	Effects on differentiation	Ref
CircHUWE1	<i>Bos taurus</i>	Myoblast	Sponge miR-29b	AKT signaling pathway	Inhibit myoblast differentiation	36
CircLMO7	<i>Bos taurus</i>	Myoblast	Sponge miR-378a-3p	Unknown	Inhibit myoblast differentiation	102
CircRNA_33287	<i>Homo sapiens</i>	MSMSC	Sponge miR-214-3p	RUNX3	Promote osteogenic differentiation	103
CircRNA-1926	<i>Capra hircus</i>	SHF stem cell	Sponge miR-14a/b-3p	CDK19	Promote SHF stem cell differentiation	104
CircRNA-1967	<i>Capra hircus</i>	SHF stem cell	Sponge miR-93-3p	LEF1	Promote SHF stem cell differentiation	105
CircSIPA1L1	<i>Homo sapiens</i>	SCAP	Sponge miR-204-5p	ALPL	Promote osteogenic differentiation	108
Hsa_circ_0002468	<i>Homo sapiens</i>	SH-SY5Y cell	Sponge miR-561	E2F8	Promote neural differentiation	111

ADSC, adipose-derived stem cell; BCLAF1, BCL2-associated transcription factor 1; BMP2, bone morphogenetic protein 2; BMPs, bone morphogenetic proteins; BMSC, bone marrow-derived mesenchymal stem cell; CBFB, core-binding factor subunit beta; CDK19, cyclin dependent kinase 19; c-JUN, c-Jun N-terminal kinase; CTNNB1, catenin beta 1; DPSC, dental pulp stem cell; ESC: embryonic stem cell; FZD4, frizzled class receptor 4; GDF5, growth differentiation factor 5; HSC: hematopoietic stem cell; hucMSC, mesenchymal stem cells derived from human umbilical cord; IFN, interferon; Igfbp2, insulin like growth factor binding protein 2; IGF, insulin like growth factor; IGF1R, insulin like growth factor 1 receptor; IGF2, insulin-like growth factor 2; iPSC, induced pluripotent stem cell; Itga5, integrin alpha 5; KLF4, kruppel like factor 4; LEF1, lymphoid enhancer binding factor 1; MDM2, murine double minute 2; MEF2C, myocyte enhancer factor 2C; MSMSC, maxillary sinus membrane stem cell; MyHC, myosin heavy chain; PDLSC, periodontal ligament stem cell; PI3K, phosphatidylinositol 3-kinase; PTBP1, polypyrimidine tract-binding protein 1; PTEN, phosphatase and tensin homolog; RBPJ, recombination signal-binding protein for immunoglobulin kappa J region; RUNX1, runt-related transcription factor 2; RUNX2, runt-related transcription factor 2; RUNX3, runt-related transcription factor 3; SCAP, stem cell from apical papilla; SHF, secondary hair follicle; SMSC, skeletal muscle satellite cell; STFs, stemness transcription factors; TCF12, transcription factor 12; VEGF, vascular endothelial growth factor; WNT5B: wnt family member 5B; ZEB1, zinc finger e-box binding homeobox 1.

proliferation and differentiation. For example, circSNX29, circFUT10, circMYL1 and circEch1 can induce myoblast differentiation while reducing myoblast proliferation.^{46–48,101} In contrast, circHUWE1 and circLMO7 can facilitate myoblast proliferation and inhibit myoblast differentiation at the same time.^{36,102}

The effects of circRNAs on the differentiation of other stem cells

In maxillary sinus membrane stem cells (MSMSCs), circRNA_33287 can facilitate osteogenic differentiation and increase ectopic bone formation *in vivo*. The underlying mechanism is that circRNA_33287 can sponge miR-214-3p and increase the expression of RUNX3.¹⁰³ In secondary hair follicle (SHF) stem cells, circRNAs act as molecular sponges for miRNAs to regulate differentiation. This can provide new method for the treatment of hair loss. During the differentiation of goat SHF stem cells into the hair follicle lineage, circRNA-1926 can promote cyclin dependent kinase 19 (CDK19) expression by sponging miR-14a/b-3p.¹⁰⁴ CircRNA-1967 also participates by sponging miR-93-3p to enhance lymphoid enhancer binding factor 1 (LEF1) expression.¹⁰⁵ Many circRNAs are upregulated during the differentiation of epidermal stem cells (EpSCs) to keratinocytes, as shown by RNA-seq analysis. Compared to host genes, upregulated circRNAs have more binding sites for argonaute 2 (AGO2), and these circRNAs may function as

miRNA sponges. One study also showed that circZNF91 contains 24 target sites for miR-23b-3p, which is essential for keratinocyte differentiation. Moreover, upregulated circRNAs are unlikely to be regulated by DNA methylation.¹⁰⁶ During the osteogenic differentiation of stem cells from apical papilla (SCAPs), 333 upregulated circRNAs and 317 downregulated circRNAs were identified by RNA-sequencing. In addition, 10 circRNAs, 21 miRNAs and mRNAs may construct competing endogenous RNA networks.¹⁰⁷ By targeting miR-204-5p, circSIPA1L1 upregulates ALPL expression and promotes osteogenic differentiation.¹⁰⁸ NSCs can differentiate into neurons, astrocytes and oligodendrites, promoting neural regeneration and repairing damage in the brain.¹⁰⁹ An analysis showed that 12 circRNAs might play regulatory roles during NSC differentiation and suggested the underlying mechanisms of circRNA-mRNA in this process.¹¹⁰ Hsa_circ_0002468 can promote neural differentiation in human neuroblastoma differentiation model SH-SY5Y cells by sponging miR-561, and miR-561 can facilitate the expression of E2F transcription factor 8 (E2F8).¹¹¹ The specific roles of circRNAs in stem cell differentiation were showed in Table 2.

The effects of circRNAs on stem cell migration

Cell migration is fundamental for development and participates in certain physiological processes, such as the immune response, wound repair and tissue homeostasis.¹¹² In

stem cells, migration is an inherent ability, similar to self-renewal and differentiation. Stem cell migration is a basic characteristic that is necessary to execute their functions. Enhancement or inhibition of this process can provide insights for the treatment of many diseases.¹¹³ For example, during wound healing, ADSCs can be recruited promptly to wound sites due to their intense migratory ability. Then, ADSCs begin to differentiate into dermal fibroblasts (DFs), endothelial cells, and keratinocytes.¹¹⁴ Several studies have confirmed that circRNAs can alter stem cell migration. In ADSCs, circ_0004303 was upregulated after ADSCs were treated with adenocarcinoma gastric cell (AGS)-derived exosomes. Increased circ_0004303 can sponge miR-148a-3p to increase the expression of activated leukocyte cell adhesion molecule (ALCAM), which is essential for migration and invasion. Therefore, ADSC migration can be enhanced by circ_0004303.¹¹⁵ In PDLSCs, circMAP3K11 can accelerate proliferation and migration, but inhibit apoptosis under inflammatory conditions. By sponging miR-511-3p, the expression of TLR4 is elevated; therefore, circMAP3K11 plays a positive role in the migration of PDLSCs.²¹ CDR1as can also contribute to PDLSC migration by maintaining the levels of stemness-related genes. CDR1as sponges miR-7 and increases KLF4 expression to improve PDLSC stemness, including migration and multi-differentiation. Moreover, the RBP hnRNPM can bind to CDR1as and regulate this process.⁷⁵ In hepatocellular carcinoma cancer stem cells (CSCs), circZKSCAN1 can negatively regulate stemness which migration was included. CircZKSCAN1 can bind to an RBP called FMRP and inhibit the expression of CCAR1, which can activate the Wnt signaling pathway. In addition, the RNA-splicing protein Quaking 5 (Qki5) can enhance this process by promoting the production of circZKSCAN1.¹¹⁶ Therefore, circRNAs may be therapeutic targets for controlling stem cell migration. However, the relationship between circRNAs and stem cell migration remains unclear.

CircRNAs regulate the aging and apoptosis in stem cells

Aging and apoptosis are normal life processes and are intricately linked with growth and development. These processes are irreversible, but we can accelerate or alleviate these processes to achieve some therapeutic goals. Therefore, it is necessary to understand the intrinsic mechanisms of aging and apoptosis. Although relevant studies have made significant progress, the roles of circRNAs in aging and apoptosis remain unclear. Here, we summarize previous studies on the effects of circRNAs on these processes.

The effects of circRNAs on stem cell aging

By using high-throughput RNA sequencing, a study revealed that 4229 circRNAs were involved in age-related senescence in MSCs. Compared with those in the young group, there were 29 differentially expressed circRNAs in the aged group, of which 4 were upregulated and 25 were downregulated. Age-related circRNAs were identified in the circRNA-miRNA-mRNA interaction network. These circRNAs

may take part in some important pathways that are associated with aging.¹¹⁷ CircRNA FUT10 was elevated in aged skeletal muscle stem cells (SkMSCs). By sponging miR-365a-3p, circRNA FUT10 can increase the expression of homeobox protein Hox-A9 (HOXA9) because miR-365a-3p can also bind to the 3'-UTR of HOXA9 mRNA. HOXA9 expression is correlated with denervated muscle atrophy.¹¹⁸ Therefore, circRNA FUT10 can reduce the proliferation and differentiation of SkMSCs and facilitate SkMSC aging.¹¹⁹

The effects of circRNAs on stem cell apoptosis

As demonstrated previously, circRNAs often play positive regulatory roles in stem cell proliferation and differentiation. However, in stem cell apoptosis, the number of circRNAs that have negative regulatory roles is greater than the number of positive circRNAs at present. In PDLSCs, circMAP3K11 inhibits apoptosis by targeting miR-511-3p to increase TLR4 expression in an inflammatory microenvironment.²¹ In BMSCs, circPVT1 can reduce apoptosis by sponging miR-21-5p, which mediates the Smad7/TGFBeta signaling pathway. And circPVT1 overexpression can attenuate steroid-induced osteonecrosis of the femoral head in rats.²⁹ In addition, circ_0000020 can also decrease BMSC apoptosis.⁵³ The circRNA AFF4 represses apoptosis in MC3T3-E1 cells by sponging miR-7223-5p.⁴² In hucMSCs, CDR1as can also reduce the apoptosis by maintaining the levels of the STFs.³⁰ In myoblasts, circRNAs typically sponge miRNAs to regulate apoptosis. For example, circHUWE1 can sponge miR-29b to activate the AKT signaling pathway and reduce bovine myoblast apoptosis.³⁶ In addition, circINSR, circRILPL1 and circCPE suppress myoblast apoptosis by sponging miR-34a, miR-145, and miR-138, respectively.^{38–40} In addition to the negative regulatory effect of circRNAs on stem cell apoptosis, proliferation or differentiation is usually promoted within the same cell. These factors contribute to the development of stem cells. CircRNAs can also promote stem cell apoptosis. CircRNA_25487 can sponge miR-134-3p to upregulate the expression of P21, thereby facilitating BMSC apoptosis. Excessive circRNA_25487 inhibited bone repair in trauma-induced osteonecrosis of femoral head.⁴⁵ In PDLSCs, circCDK8 overexpression induces autophagy and apoptosis through mTOR signaling.⁷⁶ Hsa-circ_0003420 can target the mRNA of insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) to decrease IGF2BP1 expression. This decrease results in apoptosis in acute myeloid leukemia stem cells. Moreover, hsa-circ_0003420 inhibits the characteristics of leukemia tumor stem cells.¹²⁰ Therefore, targeting circRNAs to alter stem cell apoptosis may be a useful therapeutic strategy.

CircRNAs regulate the aberrant changes in stem cells

CircRNAs can alter the malignant behavior of CSCs

In recent years, circRNAs have been shown to be important in the formation and progression of cancer, especially hepatocellular carcinoma, gastric carcinoma and colorectal cancer, which reminds us of the importance of circRNAs in cancer diagnosis and therapy.¹²¹ CSCs, which

possess self-renewal ability, are the main reason for cancer recurrence.¹²² Thus, CSCs are a key target for treating cancer. CircRNAs also play roles in CSCs and alter the malignant behavior of CSCs. Zhao et al found that circATP5B can aggravate the proliferation of glioma stem cells (GSCs) by acting as a sponge of miR-185-5p. MiR-185-5p increases the expression of homeobox B5 (HOXB5), which can activate the JAK2/STAT3 signaling pathway to promote GSC proliferation. RBP SRSF1 can promote this process by binding to circATP5B. Interestingly, HOXB5 can transcriptionally regulate SRSF1 expression, forming a feedback loop to regulate this process in GSCs.¹²³ Similarly, circCHAF1A can also take part in a feedback loop to facilitate the proliferation and tumorigenesis of TP53wt GSCs. By sponging miR-211-5p, circCHAF1A contributes to the increased expression of homeobox C8 (HOXC8), which can upregulate murine double minute2 (MDM2) expression and inhibit the antitumor effect of p53 to promote the malignant behavior of GSCs. RBP FMR1 can bind to circCHAF1A to maintain its stability and can also be regulated by HOXC8. These factors form a feedback loop among FMR1, circCHAF1A, miR-211-5p, and HOXC8 in GSCs.¹²⁴ Moreover, the U2AF2/circRNA ARF1/miR-342-3p/ISL2 feedback loop was identified in GSCs and can promote glioma angiogenesis and worsen the prognosis.¹²⁵ Therefore, circRNAs may take part in additional feedback loops to regulate GSCs or other CSCs. Surprisingly, circ-E-Cad can promote GSC tumorigenicity by encoding an E-cadherin protein variant that can activate EGFR-STAT3 signaling.¹²⁶ CircRNAs can also negatively regulate CSCs. In hepatocellular carcinoma CSCs (HCC CSCs), circZKSCAN1 can competitively bind to FMRP to inhibit the malignant behavior of HCC CSCs.¹¹⁶ CircMEG3 can restrain the growth of human liver CSCs by inhibiting telomerase activity, and this effect is dependent on HULC and Cbf5.¹²⁷

In addition, circRNAs can also alter the CSC-like properties of cancers. These properties incorporate self-renewal, proliferation, migration, and radioresistance. For example, circFAM73A enhances the CSC-like properties of gastric cancer by targeting miR-490-3p.¹²⁸ In glioma cells, circEPHB4 and circPTN can improve stem-like properties and proliferation by sponging miR-637 and miR-145-5p/miR-330-5p, respectively.^{129,130} Moreover, by sponging miR-145-5p, circPTN can promote the stemness of GSCs. CDR1as can sponge miR-641 to enhance the expression of HOXA9, thereby stimulating stemness and cisplatin chemoresistance in non-small-cell lung cancer (NSCLC).¹³¹ In contrast, BMSC-derived exosomal circ_0030167 can inhibit the stem-like properties and tumor progression of pancreatic cancer by sponging miR-338-5p and targeting the wif1/Wnt 8/beta-catenin axis.¹³²

Therefore, targeting functional circRNAs to repress CSCs or CSC-like properties in cancer is critical for developing therapeutic strategies.

CircRNAs can affect stem cell injury

Preventing the progression of cell injury is helpful for protecting normal cells. CircRNAs can alter the extent of stem cell injury. Silencing circRNA Cznf292 can attenuate oxygen-glucose deprivation/reoxygenation (OGD/R)-induced

injury in rat NSCs by sponging miR-22. Upregulated miR-22 activates the Wnt/ β -catenin and PKC/ERK pathways, which can reduce NSC injury induced by OGD/R.¹³³ Irradiation is a useful therapeutic method for tumors but can also injure normal cells and even normal stem cells. Circ-016901 contributes to irradiation-induced injury in BMSCs. Circ-016901 can sponge miR-1249-5p to improve HIPK2 expression, which can aggravate injury in BMSCs.¹³⁴ Furthermore, Wang et al showed that the surviving fraction of circRNA_014511 overexpressing BMSCs was distinctly higher than that of the control group after irradiation. Mechanistically, circRNA_014511 can sponge miR-29b-2-5p to decrease the radiosensitivity of BMSCs by altering the cell cycle and apoptosis.¹³⁵ Although there have been few studies on the effect of circRNAs on stem cell radiosensitivity, circRNAs are a new target to protect normal stem cells against radiotherapy or irradiation. We hope that additional mechanisms of stem cell injury involving circRNAs will be identified and have clinical applications.

Stem cell-derived exosomal circRNAs and their functions

The past several years have confirmed that exosomes can be broadly involved in many biological processes, including intercellular communication, immune responses, pregnancy, neurodegeneration, and cancer progression.¹³⁶ Exosomes are small extracellular vesicles approximately 40–160 nm in diameter that contain many constituents of a cell, such as nucleic acids, lipids, metabolites, and proteins. CircRNAs can also be contained in stem cell-derived exosomes and exert their functions (Table 3).

Researchers have identified several circRNAs that have significant roles in exosomes derived from ADSCs. These circRNAs include circ-Fryl, mmu_circ_0000623, mmu_circ_0000250, circAkap7, mmu_circ_0001359 and circ_100395. These circRNAs also act as sponges of miRNAs. Exosomal circ-Fryl can sponge miR-490-3p to decrease apoptosis in alveolar epithelial cells and the expression of inflammatory factors, thereby alleviating sepsis-induced lung injury.¹³⁷ Exosomal mmu_circ_0000623 can prevent liver fibrosis by sponging miR-125 to promote autophagy activation.¹³⁸ Exosomal mmu_circ_0000250 also activates autophagy in endothelial progenitor cells by sponging miR-128-3p. Autophagy contributes to wound healing in diabetic mice.¹³⁹ Moreover, exosomal circAkap7 can protect against cerebral injury by increasing ATG12-mediated autophagy and decreasing NRF2-mediated oxidative stress by sponging miR-155-5p.¹⁴⁰ By sponging miR-183-5p, exosomal mmu_circ_0001359 can increase FoxO1 signaling-mediated M2-like macrophage activation and suppress inflammatory cytokine expression to reduce airway remodeling.¹⁴¹ In addition, exosomal circ_100395 can suppress malignant transformation in NSCLC by sponging miR-141-3p.¹⁴²

In exosomes derived from BMSCs, hsa_circ_0006859 can suppress BMSC osteogenesis and promote BMSC adipogenesis by enhancing ROCK1 expression by sponging miR-431-5p.¹⁴³ By sponging miR-3677-3p, exosomal circRNA_0001236 facilitates cartilage-specific gene and protein expression, thereby alleviating cartilage

Table 3 Stem cell-derived exosomal circRNAs and their functions.

Exosomal circRNAs	Original cells	Mechanisms	Target genes or pathways	Effects	Ref
Circ-Fryl	ADSC	Sponge miR-490-3p	SIRT3	Attenuate sepsis-induced lung injury	137
Mmu_circ_0000623	ADSC	Sponge miR-125	ATG4D	Prevent liver fibrosis	138
Mmu_circ_0000250	ADSC	Sponge miR-128-3p	SIRT1	Promote wound healing in diabetic mice	139
CircAkap7	ADSC	Sponge miR-155-5p	ATG12, NRF2	Protect against cerebral ischemic injury	140
Mmu_circ_0001359	ADSC	Sponge miR-183-5p	FoxO1	Attenuate airway remodeling	141
Circ_100395	ADSC	Sponge miR-141-3p	LATS2	Inhibit the Malignant Transformation in NSCLC	142
Hsa_circ_0006859	BMSC	Sponge miR-431-5p	ROCK1	Suppress BMSC osteogenesis and promote BMSC adipogenesis	143
CircRNA_0001236	BMSC	Sponge miR-3677-3p	Sox9	Enhance chondrogenesis and suppress cartilage degradation	144
Circ_0030167	BMSC	Sponge miR-338-5p	wif1/Wnt 8/ beta-catenin axis	Inhibit the malignant behaviours pancreatic cancer cells	132
Circ-0001273	hucMSC	Unknown	Unknown	Inhibit myocardial cell apoptosis in an ischemic environment and promote MI repair	145
CircHIPK3	hucMSC	Sponge miR-421	FOXO3a	Promote the repair of ischemic muscle injury	19
CircLPAR1	DPSC	Sponge hsa-miR-31	Unknown	Promote osteogenic differentiation in recipient homotypic DPSCs	146

ADSC, adipose-derived stem cell; ATG12, autophagy-related protein 12; ATG4D, autophagy associated protein 4D antibody; BMSC, bone marrow-derived mesenchymal stem cell; DPSC, dental pulp stem cells; hucMSC, mesenchymal stem cells derived from human umbilical cord; LATS2, large tumor suppressor, homolog 2; MI, myocardial infarction; NRF2, nuclear factor erythroid-2 related factor 2; NSCLC, non-small cell lung carcinoma; ROCK1, rho associated coiled-coil containing protein kinase 1; SIRT1, sirtuin 1; SIRT3, sirtuin 3; Sox9, sex-determining region Y-box 9; wif1, wnt inhibitory factor 1.

degradation and osteoarthritis progression.¹⁴⁴ As mentioned previously, BMSC-derived exosomal circ_0030167 can also have a suppressive effect on pancreatic cancer cells by sponging miR-338-5p.¹³²

In exosomes derived from hucMSCs, circ-0001273 can inhibit myocardial cell apoptosis in an ischemic environment and facilitate myocardial infarction.¹⁴⁵ CircHIPK3 can promote the repair of ischemic muscle injury by sponging miR-421. Decreasing miR-421 expression can enhance the expression of FOXO3a, which can prevent pyroptosis in skeletal muscle cells and accelerate ischemic muscle repair.¹⁹

In addition, DPSC-derived exosomal circLPAR1 can promote osteogenic differentiation in recipient homotypic DPSCs by binding to hsa-miR-31. This binding counteracts the adverse impact of hsa-miR-31 on osteogenesis.¹⁴⁶

Concluding remarks

In brief, we summarized recent studies about the effects of circRNAs on stem cells, including biological activities, aging and apoptosis, and aberrant changes. We also introduced the biological roles of stem cell-derived exosomal circRNAs. Thanks to the development of bioinformatics, we recognize the value of circRNAs in stem cells. CircRNAs typically act as sponges of miRNAs and have their own expression patterns in stem cells. Therefore, circRNAs can also be useful

targets in diagnosis and stem cell therapy. Altering circRNA expression may change the state of stem cells to meet therapeutic requirements. However, apart from sponging miRNAs, other functions of circRNAs, such as being translated into proteins, regulating transcription and interacting with proteins are not dominant in stem cells at present. Thus, despite many prospective studies, the underlying relationships between circRNAs and stem cells are still unclear. We believe that there will be more effects of circRNAs on stem cells discovered and more mechanisms of circRNAs in the regulation of stem cells.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by The National Natural Science Foundation of China (No. 82173446).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.gendis.2022.05.015>.

References

- Chen LL. The biogenesis and emerging roles of circular RNAs. *Nat Rev Mol Cell Biol.* 2016;17(4):205–211.
- Li Z, Huang C, Bao C, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol.* 2015;22(3):256–264.
- Zhang Y, Zhang XO, Chen T, et al. Circular intronic long noncoding RNAs. *Mol Cell.* 2013;51(6):792–806.
- Liang D, Tatomer DC, Luo Z, et al. The output of protein-coding genes shifts to circular RNAs when the pre-mRNA processing machinery is limiting. *Mol Cell.* 2017;68(5):940–954.
- Guarnerio J, Bezzi M, Jeong JC, et al. Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell.* 2016;165(2):289–302.
- Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. *Mol Cell.* 2018;71(3):428–442.
- Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci U S A.* 1976;73(11):3852–3856.
- Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature.* 2013;495(7441):384–388.
- Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature.* 2013;495(7441):333–338.
- Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ, Xu RH. Circular RNA: metabolism, functions and interactions with proteins. *Mol Cancer.* 2020;19(1):172.
- Altesha MA, Ni T, Khan A, Liu K, Zheng X. Circular RNA in cardiovascular disease. *J Cell Physiol.* 2019;234(5):5588–5600.
- Jin J, Sun H, Shi C, et al. Circular RNA in renal diseases. *J Cell Mol Med.* 2020;24(12):6523–6533.
- Li J, Sun D, Pu W, Wang J, Peng Y. Circular RNAs in cancer: biogenesis, function, and clinical significance. *Trends Cancer.* 2020;6(4):319–336.
- Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration.* 2013;85(1):3–10.
- Bozdağ SC, Yüksel MK, Demirel T. Adult stem cells and medicine. *Adv Exp Med Biol.* 2018;1079:17–36.
- Zhuang Z, Jia L, Li W, Zheng Y. The emerging roles of circular RNAs in regulating the fate of stem cells. *Mol Cell Biochem.* 2021;476(1):231–246.
- Zhou J, Qiu C, Fan Z, Liu T, Liu T. Circular RNAs in stem cell differentiation: a sponge-like role for miRNAs. *Int J Med Sci.* 2021;18(11):2438–2448.
- Shen W, Sun B, Zhou C, Ming W, Zhang S, Wu X. CircFOXP1/FOXP1 promotes osteogenic differentiation in adipose-derived mesenchymal stem cells and bone regeneration in osteoporosis via miR-33a-5p. *J Cell Mol Med.* 2020;24(21):12513–12524.
- Yan B, Zhang Y, Liang C, et al. Stem cell-derived exosomes prevent pyroptosis and repair ischemic muscle injury through a novel exosome/circHIPK3/FOXO3a pathway. *Theranostics.* 2020;10(15):6728–6742.
- Wang F, Chen X, Han Y, Xi S, Wu G. circRNA CDR1as regulated the proliferation of human periodontal ligament stem cells under a lipopolysaccharide-induced inflammatory condition. *Mediat Inflamm.* 2019;2019:1625381.
- Yu B, Hu J, Li Q, Wang F. CircMAP3K11 contributes to proliferation, apoptosis and migration of human periodontal ligament stem cells in inflammatory microenvironment by regulating TLR4 via miR-511 sponging. *Front Pharmacol.* 2021;12:633353.
- Wong RSY, Cheong SK. Role of circular RNAs in determining the fate of mesenchymal stem cells. *Malays J Pathol.* 2021;43(2):241–250.
- Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. *Cell Transplant.* 2016;25(5):829–848.
- Xin W, Yuan S, Wang B, Qian Q, Chen Y. Hsa_circ_0066523 promotes the proliferation and osteogenic differentiation of bone mesenchymal stem cells by repressing PTEN. *Bone Joint Res.* 2021;10(8):526–535.
- Ren W, Yang L, Deng T, et al. Calcitonin gene-related peptide regulates FOSL2 expression and cell proliferation of BMSCs via mmu_circRNA_003795. *Mol Med Rep.* 2019;19(5):3732–3742.
- Liu N, Lu W, Qu X, Zhu C. LLL1 promotes BMSC proliferation through circRNA_0001052/miR-124-3p. *Laser Med Sci.* 2022;37(2):849–856.
- Zhang Z, Zhou H, Sun F, Han J, Han Y. Circ_FBLN1 promotes the proliferation and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by regulating let-7i-5p/FZD4 axis and Wnt/ β -catenin pathway. *J Bioenerg Biomembr.* 2021;53(5):561–572.
- Chia W, Liu J, Huang YG, Zhang C. A circular RNA derived from DAB1 promotes cell proliferation and osteogenic differentiation of BMSCs via RBPJ/DAB1 axis. *Cell Death Dis.* 2020;11(5):372.
- Hao Y, Lu C, Zhang B, Xu Z, Guo H, Zhang G. CircPVT1 up-regulation attenuates steroid-induced osteonecrosis of the femoral head through regulating miR-21-5p-mediated Smad7/TGF β signalling pathway. *J Cell Mol Med.* 2021;25(10):4608–4622.
- Yang L, Bin Z, Hui S, et al. The role of CDR1as in proliferation and differentiation of human umbilical cord-derived mesenchymal stem cells. *Stem Cell Int.* 2019;2019:2316834.
- Yue B, Yang H, Wu J, et al. circSVIL regulates bovine myoblast development by inhibiting STAT1 phosphorylation. *Sci China Life Sci.* 2022;65(2):376–386.
- Ouyang H, Chen X, Li W, Li Z, Nie Q, Zhang X. Circular RNA circSVIL promotes myoblast proliferation and differentiation by sponging miR-203 in chicken. *Front Genet.* 2018;9:172.
- Gao M, Li X, Yang Z, et al. circHIPK3 regulates proliferation and differentiation of myoblast through the miR-7/TCF12 pathway. *J Cell Physiol.* 2021;236(10):6793–6805.
- Chen B, Yu J, Guo L, et al. Circular RNA circHIPK3 promotes the proliferation and differentiation of chicken myoblast cells by sponging miR-30a-3p. *Cells.* 2019;8(2):177.
- Legnini I, Di Timoteo G, Rossi F, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. *Mol Cell.* 2017;66(1):22–37.
- Yue B, Wang J, Ru W, et al. The circular RNA circHUWE1 sponges the miR-29b-AKT3 axis to regulate myoblast development. *Mol Ther Nucleic Acids.* 2020;19:1086–1097.
- Wang X, Cao X, Dong D, et al. Circular RNA TTN acts as a miR-432 sponge to facilitate proliferation and differentiation of myoblasts via the IGF2/PI3K/AKT signaling pathway. *Mol Ther Nucleic Acids.* 2019;18:966–980.
- Shen X, Zhang X, Ru W, et al. circINSR promotes proliferation and reduces apoptosis of embryonic myoblasts by sponging miR-34a. *Mol Ther Nucleic Acids.* 2020;19:986–999.
- Shen X, Tang J, Jiang R, et al. CircRILPL1 promotes muscle proliferation and differentiation via binding miR-145 to activate IGF1R/PI3K/AKT pathway. *Cell Death Dis.* 2021;12(2):142.
- Ru W, Qi A, Shen X, et al. The circular RNA circCPE regulates myoblast development by sponging miR-138. *J Anim Sci Biotechnol.* 2021;12(1):102.
- Chen X, Ouyang H, Wang Z, Chen B, Nie Q. A novel circular RNA generated by FGFR2 gene promotes myoblast

- proliferation and differentiation by sponging miR-133a-5p and miR-29b-1-5p. *Cells*. 2018;7(11):199.
42. Mi B, Xiong Y, Chen L, et al. CircRNA AFF4 promotes osteoblast cells proliferation and inhibits apoptosis via the Mir-7223-5p/PIK3R1 axis. *Aging (Albany NY)*. 2019;11(24):11988–12001.
 43. Ma X, Xiang F, Pei Z, et al. Circ-Smad5 retards the G1/S transition of cell cycle via inhibiting the activity of wnt/lef/cyclind1 signaling in JB6 cells. *Genes Dis*. 2021;8(3):364–372.
 44. Yang B, Zang L, Cui J, Wei L. Circular RNA TTC3 regulates cerebral ischemia-reperfusion injury and neural stem cells by miR-372-3p/TLR4 axis in cerebral infarction. *Stem Cell Res Ther*. 2021;12(1):125.
 45. Zhang Y, Jia S, Wei Q, et al. CircRNA_25487 inhibits bone repair in trauma-induced osteonecrosis of femoral head by sponging miR-134-3p through p21. *Regen Ther*. 2021;16:23–31.
 46. Elnour IE, Wang X, Zhansaya T, et al. Circular RNA circMYL1 inhibit proliferation and promote differentiation of myoblasts by sponging miR-2400. *Cells*. 2021;10(1):176.
 47. Peng S, Song C, Li H, et al. Circular RNA SNX29 sponges miR-744 to regulate proliferation and differentiation of myoblasts by activating the Wnt5a/Ca²⁺ signaling pathway. *Mol Ther Nucleic Acids*. 2019;16:481–493.
 48. Li H, Yang J, Wei X, et al. CircFUT10 reduces proliferation and facilitates differentiation of myoblasts by sponging miR-133a. *J Cell Physiol*. 2018;233(6):4643–4651.
 49. Zhang M, Jia L, Zheng Y. circRNA expression profiles in human bone marrow stem cells undergoing osteoblast differentiation. *Stem Cell Rev Rep*. 2019;15(1):126–138.
 50. Li F, Wu H, Zou G, Cang D, Shen Y. Circular RNA_0062582 promotes osteogenic differentiation of human bone marrow mesenchymal stem cells via regulation of microRNA-145/CBFB axis. *Bioengineered*. 2021;12(1):1952–1963.
 51. Qin X, Jiang Q, Matsuo Y, et al. Cbfb regulates bone development by stabilizing Runx family proteins. *J Bone Miner Res*. 2015;30(4):706–714.
 52. Liu C, Liu AS, Zhong D, et al. Circular RNA AFF4 modulates osteogenic differentiation in BM-MSCs by activating SMAD1/5 pathway through miR-135a-5p/FNDC5/Irisin axis. *Cell Death Dis*. 2021;12(7):631.
 53. Zhou R, Miao S, Xu J, Sun L, Chen Y. Circular RNA circ_0000020 promotes osteogenic differentiation to reduce osteoporosis via sponging microRNA miR-142-5p to up-regulate Bone Morphogenetic Protein BMP2. *Bioengineered*. 2021;12(1):3824–3836.
 54. Huang Y, Wan S, Yang M. Circ_0067680 expedites the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells through miR-4429/CTNNB1/Wnt/ β -catenin pathway. *Biol Direct*. 2021;16(1):16.
 55. Guo Z, Xie M, Zou Y, et al. Circular RNA Hsa_circ_0006766 targets microRNA miR-4739 to regulate osteogenic differentiation of human bone marrow mesenchymal stem cells. *Bioengineered*. 2021;12(1):5679–5687.
 56. Ouyang Z, Tan T, Zhang X, et al. CircRNA hsa_circ_0074834 promotes the osteogenesis-angiogenesis coupling process in bone mesenchymal stem cells (BMSCs) by acting as a ceRNA for miR-942-5p. *Cell Death Dis*. 2019;10(12):932.
 57. Ji H, Cui X, Yang Y, Zhou X. CircRNA hsa_circ_0006215 promotes osteogenic differentiation of BMSCs and enhances osteogenesis-angiogenesis coupling by competitively binding to miR-942-5p and regulating RUNX2 and VEGF. *Aging (Albany NY)*. 2021;13(7):10275–10288.
 58. Wang X, Chen T, Deng Z, et al. Melatonin promotes bone marrow mesenchymal stem cell osteogenic differentiation and prevents osteoporosis development through modulating circ_0003865 that sponges miR-3653-3p. *Stem Cell Res Ther*. 2021;12(1):150.
 59. Zhong W, Li X, Pathak JL, et al. Dicalcium silicate microparticles modulate the differential expression of circRNAs and mRNAs in BMSCs and promote osteogenesis via circ_1983-miR-6931-Gas7 interaction. *Biomater Sci*. 2020;8(13):3664–3677.
 60. Zhu S, Zhu Y, Wang Z, et al. Bioinformatics analysis and identification of circular RNAs promoting the osteogenic differentiation of human bone marrow mesenchymal stem cells on titanium treated by surface mechanical attrition. *PeerJ*. 2020;8:e9292.
 61. Chen G, Wang Q, Li Z, et al. Circular RNA CDR1as promotes adipogenic and suppresses osteogenic differentiation of BMSCs in steroid-induced osteonecrosis of the femoral head. *Bone*. 2020;133:115258.
 62. Kang Y, Guo S, Sun Q, Zhang T, Liu J, He D. Differential circular RNA expression profiling during osteogenic differentiation in human adipose-derived stem cells. *Epigenomics*. 2020;12(4):289–302.
 63. Zhang D, Ni N, Wang Y, et al. CircRNA-vgll3 promotes osteogenic differentiation of adipose-derived mesenchymal stem cells via modulating miRNA-dependent integrin α 5 expression. *Cell Death Differ*. 2021;28(1):283–302.
 64. Wang M, Huan Y, Li X, Li J, Lv G. RUNX3 derived hsa_circ_0005752 accelerates the osteogenic differentiation of adipose-derived stem cells via the miR-496/MDM2-p53 pathway. *Regen Ther*. 2021;18:430–440.
 65. Guo Z, Zhao L, Ji S, et al. CircRNA-23525 regulates osteogenic differentiation of adipose-derived mesenchymal stem cells via miR-30a-3p. *Cell Tissue Res*. 2021;383(2):795–807.
 66. Huang X, Cen X, Zhang B, et al. The roles of circRFDW2 and circINO80 during NELL-1-induced osteogenesis. *J Cell Mol Med*. 2019;23(12):8432–8441.
 67. Huang XQ, Cen X, Sun WT, et al. CircPOMT1 and circMCM3AP inhibit osteogenic differentiation of human adipose-derived stem cells by targeting miR-6881-3p. *Am J Transl Res*. 2019;11(8):4776–4788.
 68. Zhu Y, Gui W, Lin X, Li H. Knock-down of circular RNA H19 induces human adipose-derived stem cells adipogenic differentiation via a mechanism involving the polypyrimidine tract-binding protein 1. *Exp Cell Res*. 2020;387(2):111753.
 69. Oliva-Olivera W, Coin-Aragüez L, Lhamyani S, et al. Adipogenic impairment of adipose tissue-derived mesenchymal stem cells in subjects with metabolic syndrome: possible protective role of FGF₂. *J Clin Endocrinol Metab*. 2017;102(2):478–487.
 70. Ruan ZB, Chen GC, Zhang R, Zhu L. Circular RNA expression profiles during the differentiation of human umbilical cord-derived mesenchymal stem cells into cardiomyocyte-like cells. *J Cell Physiol*. 2019;234(9):16412–16423.
 71. Gu X, Li M, Jin Y, Liu D, Wei F. Identification and integrated analysis of differentially expressed lncRNAs and circRNAs reveal the potential ceRNA networks during PDLSC osteogenic differentiation. *BMC Genet*. 2017;18(1):100.
 72. Zheng Y, Li X, Huang Y, Jia L, Li W. The circular RNA landscape of periodontal ligament stem cells during osteogenesis. *J Periodontol*. 2017;88(9):906–914.
 73. Li X, Zheng Y, Zheng Y, et al. Circular RNA CDR1as regulates osteoblastic differentiation of periodontal ligament stem cells via the miR-7/GDF5/SMAD and p38 MAPK signaling pathway. *Stem Cell Res Ther*. 2018;9(1):232.
 74. Cheng X, Yang T, Meng W, Liu H, Zhang T, Shi R. Over-expression of GDF5 through an adenovirus vector stimulates osteogenesis of human mesenchymal stem cells in vitro and in vivo. *Cells Tissues Organs*. 2012;196(1):56–67.
 75. Gu X, Li X, Jin Y, et al. CDR1as regulated by hnRNPM maintains stemness of periodontal ligament stem cells via miR-77/KLF4. *J Cell Mol Med*. 2021;25(9):4501–4515.

76. Zheng J, Zhu X, He Y, et al. CircCDK8 regulates osteogenic differentiation and apoptosis of PDLSCs by inducing ER stress/autophagy during hypoxia. *Ann N Y Acad Sci.* 2021; 1485(1):56–70.
77. Chen M, Yang Y, Zeng J, Deng Z, Wu B. circRNA expression profile in dental pulp stem cells during odontogenic differentiation. *Stem Cell Int.* 2020;2020:5405931.
78. Ji F, Zhu L, Pan J, et al. hsa_circ_0026827 promotes osteoblast differentiation of human dental pulp stem cells through the Beclin1 and RUNX1 signaling pathways by sponging miR-188-3p. *Front Cell Dev Biol.* 2020;8:470.
79. Ji F, Pan J, Shen Z, et al. The circular RNA circRNA124534 promotes osteogenic differentiation of human dental pulp stem cells through modulation of the miR-496/ β -catenin pathway. *Front Cell Dev Biol.* 2020;8:230.
80. Ge X, Li Z, Zhou Z, Xia Y, Bian M, Yu J. Circular RNA SIPA1L1 promotes osteogenesis via regulating the miR-617/Smad3 axis in dental pulp stem cells. *Stem Cell Res Ther.* 2020;11(1):364.
81. Izuogu OG, Alhasan AA, Mellough C, et al. Analysis of human ES cell differentiation establishes that the dominant isoforms of the lncRNAs RMST and FIRRE are circular. *BMC Genom.* 2018;19(1):276.
82. Yu CY, Li TC, Wu YY, et al. The circular RNA circBIRC6 participates in the molecular circuitry controlling human pluripotency. *Nat Commun.* 2017;8(1):1149.
83. Errichelli L, Dini Modigliani S, Laneve P, et al. FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. *Nat Commun.* 2017;8:14741.
84. Xia P, Wang S, Ye B, et al. A circular RNA protects dormant hematopoietic stem cells from DNA sensor cGAS-mediated exhaustion. *Immunity.* 2018;48(4):688–701.
85. Nicolet BP, Engels S, Aglialoro F, van den Akker E, von Lindern M, Wolkers MC. Circular RNA expression in human hematopoietic cells is widespread and cell-type specific. *Nucleic Acids Res.* 2018;46(16):8168–8180.
86. Yamanaka S. Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell.* 2020;27(4):523–531.
87. Barilani M, Cherubini A, Peli V, et al. A circular RNA map for human induced pluripotent stem cells of foetal origin. *EBio-Medicine.* 2020;57:102848.
88. Lei W, Feng T, Fang X, et al. Signature of circular RNAs in human induced pluripotent stem cells and derived cardiomyocytes. *Stem Cell Res Ther.* 2018;9(1):56.
89. Yu B, Li M, Han SP, Yu Z, Zhu J. Circular RNA hsa_circ_105039 promotes cardiomyocyte differentiation by sponging miR17 to regulate cyclinD2 expression. *Mol Med Rep.* 2021;24(6):861.
90. Li X, Ao J, Wu J. Systematic identification and comparison of expressed profiles of lncRNAs and circRNAs with associated co-expression and ceRNA networks in mouse germline stem cells. *Oncotarget.* 2017;8(16):26573–26590.
91. Li X, Tian G, Wu J. Novel circGFR α 1 promotes self-renewal of female germline stem cells mediated by m⁶A writer METTL14. *Front Cell Dev Biol.* 2021;9:640402.
92. Li L, Chen Y, Nie L, et al. MyoD-induced circular RNA CDR1as promotes myogenic differentiation of skeletal muscle satellite cells. *Biochim Biophys Acta Gene Regul Mech.* 2019; 1862(8):807–821.
93. Shen X, Liu Z, Cao X, et al. Circular RNA profiling identified an abundant circular RNA circTMTC1 that inhibits chicken skeletal muscle satellite cell differentiation by sponging miR-128-3p. *Int J Biol Sci.* 2019;15(10):2265–2281.
94. Yin H, Shen X, Zhao J, et al. Circular RNA CircFAM188B encodes a protein that regulates proliferation and differentiation of chicken skeletal muscle satellite cells. *Front Cell Dev Biol.* 2020;8:522588.
95. Wei Y, Tian Y, Li X, et al. Circular RNA circFNDC3AL upregulates BCL9 expression to promote chicken skeletal muscle satellite cells proliferation and differentiation by binding to miR-204. *Front Cell Dev Biol.* 2021;9:736749.
96. Shen X, Wei Y, You G, et al. Circular PPP1R13B RNA promotes chicken skeletal muscle satellite cell proliferation and differentiation via targeting miR-9-5p. *Animals.* 2021;11(8): 2396.
97. Yao R, Yao Y, Li C, et al. Circ-HIPK3 plays an active role in regulating myoblast differentiation. *Int J Biol Macromol.* 2020;155:1432–1439.
98. Chen M, Wei X, Song M, et al. Circular RNA circMYBPC1 promotes skeletal muscle differentiation by targeting MyHC. *Mol Ther Nucleic Acids.* 2021;24:352–368.
99. Wang Y, Li M, Wang Y, et al. A Zfp609 circular RNA regulates myoblast differentiation by sponging miR-194-5p. *Int J Biol Macromol.* 2019;121:1308–1313.
100. Shen X, Wei Y, Liu W, et al. A novel circular RNA circITSN2 targets the miR-218-5p/LMO7 axis to promote chicken embryonic myoblast proliferation and differentiation. *Front Cell Dev Biol.* 2021;9:748844.
101. Huang K, Chen M, Zhong D, et al. Circular RNA profiling reveals an abundant circEch1 that promotes myogenesis and differentiation of bovine skeletal muscle. *J Agric Food Chem.* 2021;69(1):592–601.
102. Wei X, Li H, Yang J, et al. Circular RNA profiling reveals an abundant circLMO7 that regulates myoblasts differentiation and survival by sponging miR-378a-3p. *Cell Death Dis.* 2017; 8(10):e3153.
103. Peng W, Zhu S, Chen J, Wang J, Rong Q, Chen S. Hsa_circRNA_33287 promotes the osteogenic differentiation of maxillary sinus membrane stem cells via miR-214-3p/Runx3. *Biomed Pharmacother.* 2019;109:1709–1717.
104. Yin RH, Zhao SJ, Jiao Q, et al. CircRNA-1926 Promotes the differentiation of goat SHF stem cells into hair follicle lineage by miR-148a/b-3p/CDK19 axis. *Animals.* 2020;10(9):1552.
105. Zhu Y, Wang Y, Zhao J, et al. CircRNA-1967 participates in the differentiation of goat SHF-SCs into hair follicle lineage by sponging miR-93-3p to enhance LEF1 expression. *Anim Biotechnol.* 2021:1–13.
106. Kristensen LS, Okholm TLH, Venø MT, Kjems J. Circular RNAs are abundantly expressed and upregulated during human epidermal stem cell differentiation. *RNA Biol.* 2018;15(2): 280–291.
107. Li Z, Li N, Ge X, et al. Differential circular RNA expression profiling during osteogenic differentiation of stem cells from apical papilla. *Epigenomics.* 2019;11(9):1057–1073.
108. Li Y, Bian M, Zhou Z, et al. Circular RNA SIPA1L1 regulates osteoblastic differentiation of stem cells from apical papilla via miR-204-5p/ALPL pathway. *Stem Cell Res Ther.* 2020; 11(1):461.
109. McKay R. Stem cells in the central nervous system. *Science.* 1997;276(5309):66–71.
110. Yang Q, Wu J, Zhao J, et al. Circular RNA expression profiles during the differentiation of mouse neural stem cells. *BMC Syst Biol.* 2018;12(Suppl 8):128.
111. Yang M, Xiang G, Yu D, et al. Hsa_circ_0002468 regulates the neuronal differentiation of SH-SY5Y cells by modulating the miR-561/E2F8 axis. *Med Sci Mon Int Med J Exp Clin Res.* 2019; 25:2511–2519.
112. Trepatt X, Chen Z, Jacobson K. Cell migration. *Compr Physiol.* 2012;2(4):2369–2392.
113. de Lucas B, Pérez LM, Gálvez BG. Importance and regulation of adult stem cell migration. *J Cell Mol Med.* 2018;22(2): 746–754.
114. Mazini L, Rochette L, Admou B, Amal S, Malka G. Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. *Int J Mol Sci.* 2020;21(4): 1306.

115. Ba L, Xue C, Li X, et al. Gastric cancer cell-derived exosomes can regulate the biological functions of mesenchymal stem cells by inducing the expression of circular RNA circ_0004303. *Stem Cell Dev.* 2021;30(16):830–842.
116. Zhu YJ, Zheng B, Luo GJ, et al. Circular RNAs negatively regulate cancer stem cells by physically binding FMRP against CCAR1 complex in hepatocellular carcinoma. *Theranostics.* 2019;9(12):3526–3540.
117. Sun H, Sun Y, Yu X, et al. Analysis of age-related circular RNA expression profiles in mesenchymal stem cells of rat bone marrow. *Front Genet.* 2021;12:600632.
118. Lu X, Liang B, Li S, Chen Z, Chang W. Modulation of HOXA9 after skeletal muscle denervation and reinnervation. *Am J Physiol Cell Physiol.* 2020;318(6):C1154–C1165.
119. Zhu M, Lian C, Chen G, Zou P, Qin BG. CircRNA FUT10 regulates the regenerative potential of aged skeletal muscle stem cells by targeting HOXA9. *Aging (Albany NY).* 2021;13(13):17428–17441.
120. Lin G, Fei Y, Zhang Y. Hsa-circ_0003420 induces apoptosis in acute myeloid leukemia stem cells and impairs stem cell properties. *Immunopharmacol Immunotoxicol.* 2021;43(5):622–631.
121. Yin Y, Long J, He Q, et al. Emerging roles of circRNA in formation and progression of cancer. *J Cancer.* 2019;10(21):5015–5021.
122. Atashzar MR, Baharlou R, Karami J, et al. Cancer stem cells: a review from origin to therapeutic implications. *J Cell Physiol.* 2020;235(2):790–803.
123. Zhao J, Jiang Y, Zhang H, et al. The SRSF1/circATP5B/miR-185-5p/HOXB5 feedback loop regulates the proliferation of glioma stem cells via the IL6-mediated JAK2/STAT3 signaling pathway. *J Exp Clin Cancer Res.* 2021;40(1):134.
124. Jiang Y, Wang Z, Ying C, Hu J, Zeng T, Gao L. FMR1/circ-CHAF1A/miR-211-5p/HOXC8 feedback loop regulates proliferation and tumorigenesis via MDM2-dependent p53 signaling in GSCs. *Oncogene.* 2021;40(24):4094–4110.
125. Jiang Y, Zhou J, Zhao J, et al. The U2AF2/circRNA ARF1/miR-342-3p/ISL2 feedback loop regulates angiogenesis in glioma stem cells. *J Exp Clin Cancer Res.* 2020;39(1):182.
126. Gao X, Xia X, Li F, et al. Circular RNA-encoded oncogenic E-cadherin variant promotes glioblastoma tumorigenicity through activation of EGFR-STAT3 signalling. *Nat Cell Biol.* 2021;23(3):278–291.
127. Jiang X, Xing L, Chen Y, et al. CircMEG3 inhibits telomerase activity by reducing Cbf5 in human liver cancer stem cells. *Mol Ther Nucleic Acids.* 2021;23:310–323.
128. Xia Y, Lv J, Jiang T, et al. CircFAM73A promotes the cancer stem cell-like properties of gastric cancer through the miR-490-3p/HMGA2 positive feedback loop and HNRNP-mediated β -catenin stabilization. *J Exp Clin Cancer Res.* 2021;40(1):103.
129. Jin C, Zhao J, Zhang ZP, et al. CircRNA EPHB4 modulates stem properties and proliferation of gliomas via sponging miR-637 and up-regulating SOX10. *Mol Oncol.* 2021;15(2):596–622.
130. Chen J, Chen T, Zhu Y, et al. circPTN sponges miR-145-5p/miR-330-5p to promote proliferation and stemness in glioma. *J Exp Clin Cancer Res.* 2019;38(1):398.
131. Zhao Y, Zheng R, Chen J, Ning D. CircRNA CDR1as/miR-641/HOXA9 pathway regulated stemness contributes to cisplatin resistance in non-small cell lung cancer (NSCLC). *Cancer Cell Int.* 2020;20:289.
132. Yao X, Mao Y, Wu D, et al. Exosomal circ_0030167 derived from BM-MSCs inhibits the invasion, migration, proliferation and stemness of pancreatic cancer cells by sponging miR-338-5p and targeting the Wif1/Wnt8/ β -catenin axis. *Cancer Lett.* 2021;512:38–50.
133. Cao Y, Liu H, Zhang J, Dong Y. Circular RNA cZNF292 silence alleviates OGD/R-induced injury through up-regulation of miR-22 in rat neural stem cells (NSCs). *Artif Cell Nanomed Biotechnol.* 2020;48(1):594–601.
134. Wen X, Zhang J, Yang W, et al. CircRNA-016901 silencing attenuates irradiation-induced injury in bone mesenchymal stem cells via regulating the miR-1249-5p/HIPK2 axis. *Exp Ther Med.* 2021;21(4):355.
135. Wang Y, Zhang J, Li J, Gui R, Nie X, Huang R. CircRNA_014511 affects the radiosensitivity of bone marrow mesenchymal stem cells by binding to miR-29b-2-5p. *Bosn J Basic Med Sci.* 2019;19(2):155–163.
136. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science.* 2020;367(6478):eaa06977.
137. Shen W, Zhao X, Li S. Exosomes derived from ADSCs attenuate sepsis-induced lung injury by delivery of circ-Fryl and regulation of the miR-490-3p/SIRT3 pathway. *Inflammation.* 2022;45(1):331–342.
138. Zhu M, Liu X, Li W, Wang L. Exosomes derived from mmu_circ_0000623-modified ADSCs prevent liver fibrosis via activating autophagy. *Hum Exp Toxicol.* 2020;39(12):1619–1627.
139. Shi R, Jin Y, Hu W, et al. Exosomes derived from mmu_circ_0000250-modified adipose-derived mesenchymal stem cells promote wound healing in diabetic mice by inducing miR-128-3p/SIRT1-mediated autophagy. *Am J Physiol Cell Physiol.* 2020;318(5):C848–C856.
140. Xu L, Ji H, Jiang Y, et al. Exosomes derived from circAkap7-modified adipose-derived mesenchymal stem cells protect against cerebral ischemic injury. *Front Cell Dev Biol.* 2020;8:569977.
141. Shang Y, Sun Y, Xu J, et al. Exosomes from mmu_circ_0001359-modified ADSCs attenuate airway remodeling by enhancing FoxO1 signaling-mediated M2-like macrophage activation. *Mol Ther Nucleic Acids.* 2020;19:951–960.
142. Zhang C, Cao J, Lv W, Mou H. CircRNA_100395 carried by exosomes from adipose-derived mesenchymal stem cells inhibits the malignant transformation of non-small cell lung carcinoma through the miR-141-3p-LATS2 axis. *Front Cell Dev Biol.* 2021;9:663147.
143. Zhi F, Ding Y, Wang R, Yang Y, Luo K, Hua F. Exosomal hsa_circ_0006859 is a potential biomarker for postmenopausal osteoporosis and enhances adipogenic versus osteogenic differentiation in human bone marrow mesenchymal stem cells by sponging miR-431-5p. *Stem Cell Res Ther.* 2021;12(1):157.
144. Mao G, Xu Y, Long D, et al. Exosome-transported circRNA_0001236 enhances chondrogenesis and suppress cartilage degradation via the miR-3677-3p/Sox9 axis. *Stem Cell Res Ther.* 2021;12(1):389.
145. Li CX, Song J, Li X, Zhang T, Li ZM. Circular RNA 0001273 in exosomes derived from human umbilical cord mesenchymal stem cells (UMSCs) in myocardial infarction. *Eur Rev Med Pharmacol Sci.* 2020;24(19):10086–10095.
146. Xie L, Guan Z, Zhang M, et al. Exosomal circLPAR1 promoted osteogenic differentiation of homotypic dental pulp stem cells by competitively binding to hsa-miR-31. *BioMed Res Int.* 2020;2020:6319395.