Roles of circular RNAs in osteogenic differentiation of bone marrow mesenchymal stem cells (Review)

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Received February 22, 2022; Accepted May 10, 2022

DOI: 10.3892/mmr.2022.12743

Abstract. Bone marrow mesenchymal stem cells (BMSCs) can differentiate into osteoblasts, chondrocytes, adipocytes and even myoblasts, and are therefore defined as pluripotent cells. BMSCs have become extremely important seed cells in gene therapy, tissue engineering, cell replacement therapy and regenerative medicine due to their potential in multilineage differentiation, self-renewal, immune regulation and other fields. Circular RNAs (circRNAs) are a class of non-coding RNAs that are widely present in eukaryotic cells. Unlike standard linear RNAs, circRNAs form covalently closed continuous loops with no 5' or 3' polarity. circRNAs are abundantly expressed in cells and tissues, and are highly conserved and relatively stable during evolution. Numerous studies have shown that circRNAs play an important role in the osteogenic differentiation of BMSCs. Further studies on the role of circRNAs in the osteogenic differentiation of BMSCs can provide a new theoretical and experimental basis for bone tissue engineering and clinical treatment.

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Abbreviations: BMSC, bone marrow mesenchymal stem cell; circRNA, circular RNA; SONFH, steroid-induced osteonecrosis of the femoral head; Runx2, runt-related transcription factor 2; BMP2, bone morphogenetic protein 2; OPN, osteopontin; OP, osteoporosis; VEGF, vascular endothelial growth factor; OVX, ovariectomized; LLLI, low-level laser irradiation; PTEN, phosphatase and tensin homolog deleted on chromosome 10

Key words: BMSC, circRNA, osteogenic differentiation, bone defects, treatment

1. Introduction

Bone marrow mesenchymal stem cells (BMSCs) were first discovered in the bone marrow by Friedenstein *et al* (1). Due to the multi-directional differentiation potential of BMSCs, under specific induction conditions, they can develop into osteoblasts, adipocytes, chondrocytes and osteoblasts fibroblasts, and even differentiate into myoblasts (2-4), and are therefore defined as pluripotent cells. In addition, BMSCs can also undergo self-renewal and generate immunomodulatory responses (5,6). Studies have shown that BMSCs are capable of differentiating into multiple lineages, including tissues other than their origin, such as neurons, hepatocytes and skeletal muscle cells (7-10). BMSCs are easy to obtain, easy to expand *in vitro* and still have good differentiation potential after they are isolated from adult bone marrow (11). BMSCs have become extremely important seed cells in gene therapy, tissue engineering, cell replacement therapy and regenerative medicine due to their potential for multi-directional differentiation, self-renewal and immune regulation.

Circular RNA (circRNA) is a large class of non-coding RNA (ncRNA) that is ubiquitous in eukaryotic cells. Unlike normative linear RNAs, circRNAs form covalently closed continuous loops without 5' or 3' polarity (12). circRNAs are abundantly expressed in cells and tissues, are highly conserved in evolution and are relatively stable, and they are generally considered to be by-products of mis-splicing or messenger RNA (mRNA) processes (13). With the rapid development of high-throughput RNA sequencing (RNA-Seq) technology and bioinformatics methods, a large number of circRNAs have been discovered and identified in a number of species; for example, circ_28313, circ_0016624, circ_0006393, circ_0076906 and circ_0048211. Numerous studies have shown that circRNAs play an important role in the osteogenic differentiation of BMSCs (11,13). Further research on the role of circRNAs in the osteogenic differentiation of BMSCs can provide a new theoretical and experimental basis for bone tissue engineering and clinical treatment.

2. Biological functions of circRNAs

In 1970s, Sanger *et al* (14) and Hsu and Coca-Prados (15) first discovered circRNA in plants and eukaryotes using electron microscopy. Subsequently, PCR amplification and sequencing confirmed the expression of circRNAs in humans (16). With the development of RNA-seq and bioinformatics, thousands of circRNAs have been discovered in different species (17), with each of circRNA regulating multiple biological processes, such as CDR1 and non-coding RNA ANRIL.

According to the genomic origin and structural characteristics of circRNAs, they are mainly divided into three types: Exonic circRNA, exon-intron circRNA and intronic circRNA (18,19). The production of circRNAs is a highly complex biological process and they are produced by different cyclization mechanisms. Usually, eukaryotic pre-mRNA catalyzes the removal of introns and ligates exons by a spliceosome mechanism to form linear RNA transcripts with 5' or 3' polarity (20). Unlike the normative splicing of linear RNA, most circRNAs are produced by a backsplicing process that does not follow the 5'-3' order of the specification (20,21). Exon cyclization between the downstream 5' splice site (splicing donor) and the upstream 3' splice site (shear acceptor) in the same pre-mRNA yields a circular product (circRNA) without a terminal structure [e.g., a 5' cap or polyadenylation (poly A) tail] (18,22,23). In 2013, Jeck *et al* (18) proposed a model for two exon cyclization mechanisms. One mechanism is known as lariat-driven circularization or exon skipping. The partially folded pre-mRNA transcript brings the original non-adjacent exons close to other exons, causing exon skipping, creating overlapping regions, and forming a lasso intermediate containing exons and introns. The intron in the lasso is removed, eventually producing an exon circRNA. In general, introns located between cyclized exons are spliced out, and in some cases are not spliced to form exon-intron circRNAs (24). Another mechanism is known as intron-pairing driven circularization or direct backsplicing. This model forms a circular structure by linking the downstream splice donor to the upstream splice acceptor by ALU (identified as the canonical ALU repeat) complementarity across the flanking intron or base assignment of other RNA secondary structures. Intron circRNAs produced by intron lasso are resistant to degradation by de-branching enzymes (18,24). In distinguishing intron circRNAs from exon circRNAs, intron circRNAs contain a unique 2'-5' linkage, which is formed by sequences near the 7 nt GU-rich 5' splice site and close to 11 nt-rich C-sequences at branch point sites (25). Fig. 1 shows a schematic illustration for the biogenesis of circRNAs.

In addition, a recent study confirmed another circRNA biogenesis model acting through RNA-binding proteins (RBPs). In this case, the alternative splicing factor protein quaking and muscleblind protein bring the two flanking intron sequences together by binding to some of the circRNAs flanking the intron and forming a bridge, promoting the circularization to form circRNAs (26,27). This mechanism is similar to the intron-pairing-driven circularization pathway, except that RBPs regulate adjacent splice sites instead of the direct base pairing between complementary motifs observed in the intron-pairing-driven model.

New evidence suggests that circRNAs can act as microRNA (miR/miRNA) sponges, interact with RBPs and regulate gene transcription, and that certain circRNAs can be translated into proteins or peptides (18,24,25,28-33). Therefore, circRNAs mainly have the following functions: i) miRNA sponges. miRNAs are a class of linear non-coding RNAs that bind directly to target mRNAs through base-pair pairing, silencing or degrading target mRNAs, thereby participating in the regulation of pathological and physiological processes (34). circRNAs have miRNA sponge binding sites that serve as competitive endogenous RNAs to inhibit miRNA binding to targets and thereby inhibit mRNA translation. ii) Interact with RBPs. circRNAs bind to RBPs to form RNA-protein complexes (RPCs). These RPCs can regulate RBPs and then interact with linear RNA to exert biological functions (35,36). iii) Regulate gene transcription. circRNAs are abundantly present in the nucleus and can bind to RBPs, especially transcription-related factors, including RNA polymerase II and transcription factors, and recruit them to the parental gene, thereby affecting the expression of the parental gene and regulating the transcription process (37). iv) Translate into proteins or peptides. Previous studies have found that circRNAs also have the function of translating proteins. When synthetic circRNAs contain an internal ribosome entry site sequence that is efficiently translated, circRNAs bind directly to the ribosome and are translated in eukaryotic cells (26,38). Another study confirmed that natural eukaryotic endogenous circRNAs can drive protein translation through methylation of adenosine N6, suggesting that circRNAs have a function to encode proteins (39,40).

3. Associations between circRNAs and BMSCs

In humans, osteoblasts, which are involved in bone formation, are inseparable from the differentiation of bone marrow mesenchymal stem cells. Studies have shown that circRNAs play an important role in the osteogenic differentiation of BMSCs, and different circRNAs can either promote or inhibit the osteogenic differentiation of BMSCs (41). Fu *et al* (42) found differentially expressed circRNAs in patients with osteoporosis (OP), and the study identified 237 upregulated and 279 downregulated circRNAs, which also confirmed that the role of circRNAs in the osteogenic differentiation of BMSCs is important in the process. Another study found that circRNAs were differentially expressed in patients with traumatic femoral head necrosis, and identified 234 upregulated and 148 downregulated circRNAs (43). Chen *et al* (44) found that circRNAs, such as circ_28313, circ_0016624, circ_0006393, circ_0076906 and circ_0048211, were differentially expressed in patients with OP and play an important role in the differentiation, proliferation and apoptosis of BMSCs.

Zhang *et al* (43) found that circRNA_25487 was significantly upregulated in the peripheral blood of patients with traumatic femoral head necrosis according to reverse transcription-quantitative PCR, and further experiments found that circRNA_25487 could function as an miR-134-3p sponge. Inhibiting the expression of circRNA_25487 and promoting the expression of miR-134-3p can promote cell proliferation and invasion, and inhibit the apoptosis of BMSCs and osteoclast-like cells (43). p21 is a target of miR-134-3p.

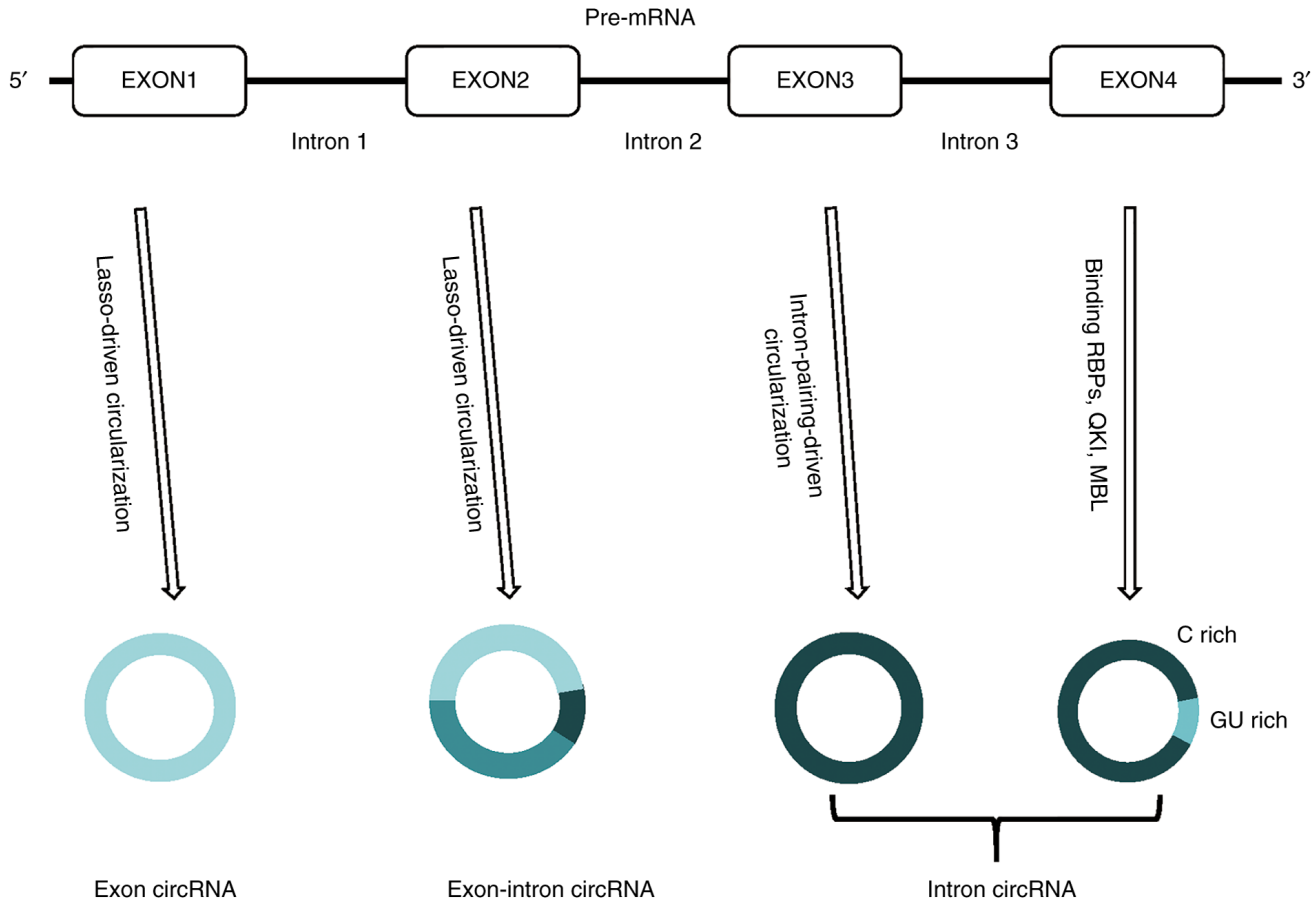

Figure 1. Schematic illustration of the biogenesis of circRNAs. pre-mRNA, pre-messenger RNA; RBP, RNA-binding protein; circRNA, circular RNA; QKI, protein quaking; MBL, muscleblind protein.

circRNA_25487 acts as an miR-134-3p sponge to upregulate p21 expression, thereby inhibiting bone repair in traumatic femoral head necrosis (43). Zhang *et al* (45) found that circIGSF11 inhibited the osteogenic differentiation process of BMSCs, while silencing circIGSF11 promoted osteoblast differentiation and increased the expression of miR-199b-5p, which also indicated that circRNA-miRNA interactions contribute to the osteogenic differentiation of BMSCs (45). This study provides a potential approach for the treatment of OP.

Steroid-induced osteonecrosis of the femoral head (SONFH) is a common orthopedic disease. Chen *et al* (46) showed that there are differentially expressed circRNAs in patients with SONFH. Bioinformatics analysis found that the expression of circRNA CDR1as was upregulated, and further experiments found that it may play a key role in the adipogenic/osteogenic differentiation of SONFH-BMSCs through the CDR1as-miR-7-5p-WNT5B axis. Knockdown of CDR1as promoted osteogenic differentiation and inhibited adipogenic differentiation of BMSCs, while overexpression of CDR1as inhibited osteogenic differentiation and promoted adipogenic differentiation of BMSCs (46). This study provides new insights into the molecular mechanism of the osteogenic/adipogenic differentiation of SONFH-BMSCs and the diagnosis and treatment of SONFH. Phosphatase and tensin homolog (PTEN) is a classic tumor suppressor that inhibits phosphatidylinositol 3-phosphate kinase (PI3K)/AKT signaling (47). Another study found that the expression of circUSP45 was increased in patients with glucocorticoid-induced osteonecrosis of the femoral head (GIONFH) (48). Overexpression of circUSP45 decreased the expression of osteogenic genes and inhibited the proliferation of BMSCs, and further experiments found that circUSP45 could directly interact with miR-127-5p. miR-127-5p regulates osteogenesis with its target PTEN (48). circUSP45 decreases the osteogenic differentiation of GIONFH by sponging miR-127-5p through the PTEN/AKT signaling pathway. Differentially expressed circRNAs were found in elderly patients with OP, and through further experiments, it was found that circRNA008876 can play a biological role as an miR-150-5p sponge (49), which provides a potential biomarker and therapeutic target for senile OP. A previous study showed that Shh coreceptor growth arrest-specific 1 (GAS1) is expressed in mesenchymal cells, and in a GAS1-deficient mouse model, mice have abnormal dentition (50). Another study has also shown that inhibiting the expression of circ_0003865 in patients with OP can promote the osteogenic differentiation of BMSCs, and that circ_0003865 regulates the expression of the GAS1 gene by sponging miR-3653-3p (51). A summary of the effect of the inhibition of circRNAs on the osteogenic differentiation of BMSCs is shown in Table I.

Although a number of circRNAs play an inhibitory role in the osteogenic differentiation of BMSCs, other circRNAs that can promote the osteogenic differentiation of BMSCs have been discovered following continuous exploration,

Table I. Effect of the inhibition of circRNAs on the osteogenic differentiation of bone marrow stem cells.

circRNA	Target	Signaling pathway/axis	Function	(Refs.)
circRNA_25487	miR-134-3p	circRNA_25487-miR-134-3p-p21 axis	Inhibit cell proliferation and promote apoptosis	(43)
circIGSF11	miR-199b-5p	circIGSF11-miR-199b-5p-GSK-3β	Inhibit osteogenesis	(45)
circRNA CDR1as	miR-7-5p	CDR1as-miR-7-5p-WNT5B axis	Inhibit osteogenesis and promote adipogenesis	(46)
circUSP45	miR-127-5p	circUSP45-miR-127-5p-PTEN/AKT	Inhibit cell proliferation	(48)
circRNA008876	miR-150-5p	circRNA008876-miR-150-5p-mRNA axis	Inhibit cell proliferation	(49)
circ_0003865	miR-3653-3p	circ_0003865-miR-3653-3p-GAS1 axis	Inhibit osteogenesis	(51)

circRNA, circular RNA; mRNA, messenger RNA; miR, microRNA.

such as circATRNL1, circRNA-016901, hsa_circ_0000219, hsa_circ_0004588 and hsa_circ_0005936 (52).

Runt-related transcription factor 2 (Runx2) belongs to the Runx family, with the DNA-binding domain runt, and consists of Runx1, Runx2 and Runx3 (53). Studies have shown that Runx2 plays an important role in the osteogenic differentiation of BMSCs (54). Ji *et al* (55) found that the expression of hsa_circ_0006215 was decreased in the BMSCs of patients with OP. Lentiviral experiments found that overexpression of hsa_circ_0006215 promoted the osteogenic differentiation of BMSCs, and hsa_circ_0006215 combined with miRNA-942-5p to regulate the expression of RUNX2 and vascular endothelial growth factor (VEGF) in BMSCs (55). The results of this study suggest that hsa_circ_0006215 plays an important role in osteogenesis and may be a new target for the treatment of elderly OP. A recent study showed that exosome-modified circ-Rtn4 could attenuate TNF-α-induced cytotoxicity and apoptosis in murine MC3T3-E1 cells, and miR-146a was identified as a target of circ-Rtn4 (56). These findings suggest that circ-Rtn4 may serve as a new candidate for the treatment of OP. Previous studies have found that low-level laser irradiation (LLLI) can promote osteoblast proliferation and bone repair (57,58), LLLI can promote BMSC proliferation (59,60), and LLLI can increase the expression of VEGF, thereby inducing the angiogenesis necessary for wound healing (61). Liu *et al* (62) showed that LLLI can regulate the proliferation of BMSCs, and circRNA_0001052 can regulate the proliferation of BMSCs through the Wnt4/β-catenin pathway as an miR-124-3p sponge. The study also demonstrated that circRNA_0001052 plays an important role in the proliferation of BMSCs in response to LLLI treatment, which provides a potential clinical application for the treatment of OP.

Articular cartilage damage is one of the main pathological changes in osteoarthritis, and cartilage repair is the key to solving osteoarthritis. Zheng *et al* (63) found that circATRNL1 (hsa_circ_0020093) was highly expressed during the chondrogenic differentiation of BMSCs, and also found that the chondrogenic differentiation-related factors SRY-related HMG box 9 (SOX9), type II collagen (COL2) and aggrecan were highly expressed. Overexpression of circATRNL1 enhanced the proliferation of BMSCs and simultaneously enhanced the expression of SOX9, COL2 and aggrecan, as well as the degree of chondrogenic differentiation of BMSCs, and miR-338-3p was its target (63). This study demonstrated that circATRNL1 promotes the cartilage differentiation of BMSCs by regulating miR-338-3p, which provides new insights into cartilage repair. A previous study has shown that circ-016901 promotes the proliferation of irradiation-induced BMSCs and attenuates irradiation-induced apoptosis by regulating the miR-1249-5p/homeodomain interacting protein kinase 2 axis (64). Li *et al* (65) found that circ45842081|58485447, circ43400193|43461320, circ18349845|183537970 and circ106417736|106434369 acted together on miR-326-5p, and that overexpression of miR-326-5p could promote the osteogenic differentiation of BMSCs while inhibiting the adipogenic differentiation. Another study showed that lentivirus-mediated small interfering RNA has_circ_0000885 plasmid transfection into BMSCs and an osteoclast co-culture system could promote BMSC cell proliferation, inhibit apoptosis and promote osteogenic differentiation (66). This provides a new target for the treatment of patients with OP. There are differentially expressed circRNAs in postmenopausal OP patients, and further research found that hsa_circ_0009127, hsa_circ_0090759, hsa_circ_0058392, hsa_circ_0090247 and hsa_circ_0049484 were involved in the regulation of autophagy, and the PI3K-AKT, FoxO and MAPK signaling pathways, thereby regulating the osteogenic differentiation process of BMSCs (42). BMSCs were isolated from ovariectomized (OVX) mice and normal mice, and further experiments found that circRNA_0020 and circRNA_3832 were downregulated in the OVX mice, and that overexpression of circRNA_0020 and circRNA_3832 could promote the osteogenic differentiation of BMSCs and promote cell proliferation (67).

A previous study has shown that the expression of osteopontin (OPN) is increased in osteoarthritis, that it accelerates the renewal and remodeling of subchondral bone in osteoarthritis, and that it mediates the degeneration of articular cartilage induced by subchondral bone metabolism (68). Liu *et al* (69) found that circ_0005564 was highly expressed and decreased the mRNA expression of RUNX2 and OPN during the osteogenic differentiation of BMSCs, and that knockdown of circ_0005564 inhibited osteoblast differentiation in BMSCs. Another recent study (70) found that circ-DAB1

Table II. Effect of the promotion of circRNAs on the osteogenic differentiation of bone marrow stem cells.

circRNA	Target	Signaling pathways/axis	Function	(Refs.)
hsa_circ_0006215	miR-942-5p	hsa_circ_0006215-miRNA-942-5p-RUNX2/VEGF axis	Promote osteogenesis	(55)
circ-Rtn4	miR-146a	circ-Rtn4-miR-146a-Bax protein/caspase-3 axis	Inhibit apoptosis and attenuate cytotoxicity	(56)
circRNA_0001052	miR-124-3p	circRNA_0001052-miR-124-3p-Wnt4/β-catenin	Promote cell proliferation and promote healing	(62)
circATRNL1	miR-338-3p	circATRNL1-miR-338-3p-SOX9/COL2/aggrecan axis	Promote cell proliferation	(63)
circ-016901	miR-1249-5p	circ-016901-miR-1249-5p-HIPK2 axis	Promote cell proliferation and inhibit apoptosis	(64)
circ45842081058485447	miR-326-5p	circRNA-miR-326-5p-mRNA axis	Promote osteogenesis	(65)
circ_0005564	RUNX2, OPN	circ_0005564-RUNX2/OPN axis	Promote osteogenesis	(69)
circDAB1	miR-1270, miR-944	circDAB1-miR-1270/miR-944-NOTCH/RBPJ	Promote cell proliferation and osteogenesis	(70)
circ_1983	miR-6931	circ_1983-miR-6931-Gas7	Promote osteogenesis	(71)
circPVT1	miR-21-5p	circPVT1-miR-21-5p-Smad7/TGFβ	Promote cell proliferation and inhibit apoptosis	(72)
circ_0000020	miR-142-5p	circ_0000020-miR-142-5p-BMP2/SMAD	Inhibit apoptosis and promote osteogenesis	(73)

circRNA, circular RNA; mRNA, messenger RNA; miR, microRNA; Runx2, runt-related transcription factor 2; BMP2, bone morphogenetic protein 2; VEGF, vascular endothelial growth factor; OPN, osteopontin; BMP, bone morphogenetic protein; SOX9, SRY-related HMG box 9; COL2, type II collagen; HIPK2, homeodomain interacting protein kinase 2; RBPJ, recombination signal binding protein for immunoglobulin-κ-J region.

(hsa_circ_0113689) was significantly upregulated during the osteogenic differentiation of human BMSCs, and that overexpression of circ-DAB1 could promote the proliferation and osteogenic differentiation of BMSCs. miR-1270 and miR-944 are targets of circ-DAB1, and further experiments found that circ-DAB1 promotes the cell proliferation and osteogenic differentiation of BMSCs through the NOTCH/recombination signal binding protein for immunoglobulin-κ-J region (RBPJ) pathway (70). Zhong *et al* (71) showed that circ_1983 acts as a sponge of miR-6931 to promote the osteogenic differentiation of BMSCs. Hao *et al* (72) found that circPVT1 was decreased in the femoral head of rats with SIONFH and glucocorticoid (GC)-treated BMSCs, whereas miR-21-5p was upregulated, and overexpression of circPVT1 attenuated GC-induced BMSC apoptosis and cell viability inhibition. circPVT1 acts as a sponge for miR-21-5p (72). Bone morphogenetic protein 2 (BMP2) plays an important role in osteogenesis. It was found that circ_0000020 was able to regulate the expression of BMP2 (73); circ_0000020 is upregulated during osteogenic differentiation, while the expression of miR-142-5p is significantly decreased (73). Silencing circ_0000020 inhibits osteogenic differentiation and promotes apoptosis, and inhibits the activity and mineralization of alkaline phosphatase. circ_0000020 can directly act on miR-142-5p (73). In conclusion, circ_0000020 positively regulates the osteogenic differentiation of BMSCs by regulating BMP2 expression via sponging miR-142-5p. Based on the aforementioned studies, circRNAs play a key role in the osteogenic differentiation of BMSCs. Different circRNAs can promote or inhibit the osteogenic differentiation of BMSCs, which also provides a new direction for OP and bone defect repair. The effect of the promotion of circRNAs on the osteogenic differentiation of BMSCs is shown in Table II.

4. Conclusions and perspectives

BMSCs are an important source of osteogenic seed cells in tissue engineering, and circRNAs play a key role in the osteogenic differentiation of BMSCs. Further studies on the functional specificity of circRNA target genes and the interactions between circRNAs are important for elucidating their mechanism of action. This research also provides a new theoretical and experimental basis for bone tissue engineering and clinical treatment.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Authors' contributions

JW and BY drafted the manuscript and revised the manuscript. TW, FZ, YZ, YG and XJ contributed to manuscript conception. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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