Functional role of circRNAs in osteogenesis: A review

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Functional role of circRNAs in osteogenesis: A review

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ABSTRACT

The extracellular matrixes (ECM), as well as the microenvironmental signals, play an essential role in osteogenesis by regulating intercellular pathways. Recently, it has been demonstrated that a newly identified RNA, circular RNA, contributes to the osteogenesis process. Circular RNA (circRNA), the most recently identified RNA, is involved in the regulation of gene expression at transcription to translation levels. The dysregulation of circRNAs has been observed in several tumors and diseases. Also, various studies have shown that circRNAs expression is changed during osteogenic differentiation of progenitor cells. Therefore, understanding the role of circRNAs in osteogenesis might help the diagnosis as well as treatment of bone diseases such as bone defects and osteoporosis. In this review, circRNA functions and the related pathways in osteogenesis have been discussed.

1. Introduction

Bone is a dynamic hard tissue with a highly vascular texture. Bone shapes the body, protects the internal organs, helps movement and locomotion, acts as a reservoir of growth factors, and is involved in hematopoiesis [1]. Therefore, any bone defects affect the quality of life and impose heavy costs on the healthcare services [2]. Bone minerals and the extracellular matrix (ECM) determine the physicomechanical properties of the organ [3]. It is well-determined that collagen type I is the major component of the bone ECM accounting for up to 90% of total tissue protein. Osteonectin, osteocalcin, osteopontin, fibronectin, and sialoprotein are other non-collagenous bone ECM components. In addition, polysaccharides also exist in bone ECM. Bone has a highly mineralized ECM and contains inorganic materials such as hydroxyapatite (HA), amorphous calcium phosphate (ACP), and carbonated apatite (CHA). Bone also consists of several bone-specific cells including osteocytes, osteoblasts, osteoclasts as well as osteoprogenitors [4,5].

Collagen I as a scaffold controls the mechanical structure of the bone tissue and also promotes bone remodeling. Osteonectin and osteocalcin are responsible for the formation and secretion of minerals such as HA while osteocalcin controls the mineralization process and also promotes bone remodeling. Bone minerals are responsible for bone rigidity and enhance bone turnover. Osteoprogenitors are considered as a reservoir to produce osteocytes and therefore are involved in bone maintenance. Osteoblasts are involved in osteoid calcification while osteoclasts regulate the balance between calcium and phosphate. The balance between osteoblasts and osteoclast is essential for the normal development of bone [4,5].

During developmental stages, bone-specific cells are derived from their progenitors including mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). MSCs differentiate into osteoprogenitor cells which in turn differentiate into osteoblasts and mature and functional osteocytes. HSCs are responsible for the generation of osteoclasts. Osteogenesis includes the formation of bone tissue with functional

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properties from bone-specific cells through ECM production and bone mineralization [6]. Osteogenesis occurs in three major phases including (1) cell proliferation, (2) matrix maturation, and (3) matrix mineralization. During osteoblast proliferation, procollagen I and fibronectin are highly expressed. In the matrix maturation phase, the expression of alkaline phosphatase (ALP) increased. The expression of bone-specific proteins including osteocalcin and osteopontin is the main characteristic of matrix mineralization [7]. Microenvironmental signals such as growth factors/inducers and extracellular matrix (ECM) play an essential role in osteogenesis. These growth factors are expressed in temporal and spatial order enhancing the 3D growth of cells on the ECM context to form new bone [6]. Transforming growth factor-β1 (TGF-β1), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), and bone morphogenetic proteins (BMPs) are among the most important factors that are involved in bone development and formation [2]. It has been shown that microRNAs (miRNAs, miRs) also have an important role in osteopoiesis and osteogenesis [8]. Recently, it has been demonstrated that a newly identified RNA, circular RNA, may have a role in osteogenesis [9]. In this review, we performed a literature search in *PubMed/MEDLINE*, *Web of Science*, and *Google Scholar* databases using the following keywords: "Circular RNA OR circRNA OR cRNA" AND "Osteogenesis OR osteogenic differentiation" AND "Dental stem cells OR DSCs". The search was conducted by two of the authors (first author and corresponding author) independently up to 24 September 2022. The relevant articles were classified based on the heading of this manuscript and the manuscript writing was performed by the authors.

2. CircRNAs: Biogenesis and biological functions

2.1. The biogenesis of circRNAs

Several kinds of coding and non-RNAs have been identified in eukaryotic cells. Many of them such as miRNAs and long non-coding RNAs (lncRNAs) regulate the gene expression mostly at the transcript level. A new identified RNA, circular RNA (circRNA), is also involved in the regulation of gene expression. CircRNAs are single-stranded and their ends covalently bind together to form a circular strand. Unlike miRNAs, circRNAs have higher stability and half-life as they are circular and resistant to exonucleases. Circular RNAs are usually generated by alternate splicing mechanisms including back splicing, exon skipping, and internal splicing during the processing and maturation of eukaryotic messenger RNAs (mRNAs). In addition, some of the circRNAs are derived from pre-tRNA transcripts during pre-tRNA processing. Based on their paternal transcript sequences, circRNAs are classified as follows (Fig. 1): (1) Exonic circRNAs (EcRNAs) derived from the exonic sequences of pre-mRNA transcripts, (2) Intronic circRNAs (ciRNAs) derived from the intronic sequences of pre-mRNA transcripts, (3) Exonic-intronic circRNAs (EIciRNAs) derived from both exonic and intronic sequences of pre-mRNA transcripts, and (4) TricRNAs derived from the sequences within pre-tRNA transcripts [10]. CircRNAs derived from pre-mRNA transcripts are synthesized in a process called "back splicing", a canonical alternative splicing. Back splicing recruits the spliceosome machinery of a canonical splicing process which binds to the sequences associated with 5' and 3' ends of circRNAs. The spliceosome machinery cleaves their target sites and covalently binds with 5' and 3' ends to form circRNAs [11]. The biogenesis of tricRNAs relies on the tRNA splicing endonuclease (TSEN) complex which cuts the bulge-helix-bulge (BHB) motifs on pre-tRNA transcripts [12]. Several RNA binding proteins (RBPs) have been identified to regulate the biogenesis of circRNAs including Muscleblind (MBL) and Quaking facilitating circularization [13,14], and adenosine deaminase negatively regulating the circularization process [15]. While circRNAs have a circular structure, they are more stable than other RNAs. To degrade circRNAs, a combination of 5' and 3' exonucleases, as well as endonucleases, are required as endonucleases open the circular structure and exonucleases further degrade the circRNA sequence [10].

2.2. The biological functions of circRNAs

CircRNAs are synthesized from pre-mRNA or pre-tRNA transcripts and then localized in the nucleus or cytoplasm. CircRNAs have been demonstrated to regulate gene expression from transcription to translation levels. CircRNAs mediate their function through different mechanisms: (1) They could regulate the expression of their parental genes in the nucleus by enhancing polymerase II elongation [16]. (2) On the contrary, some circRNAs may suppress the expression of their parental genes by recruiting the spliceosome machinery and promoting back splicing [17]. (3) The most important function of circRNAs is suppressing the function of miRNAs in a process called "miRNA sponge". During miRNA sponge, cytoplasmic circRNAs bind to their complementary sequences on miRNAs and prevent their ability to bind to their target sequences on mRNAs [18]. (4) CircRNAs may also bind to and change specific regulatory proteins, a process called "protein decoy". The secondary and tertiary structures of circRNAs as well as their sequences mediate circRNA-protein interaction in an appropriate cellular location. The circRNA-protein interaction suppresses the normal physiological function of the protein which is called protein decoy [19]. Specific circRNAs have binding sites for specific proteins which mediate circRNA-protein interaction under specific circumstances and usually alter gene expression [20]. (5) Some of the circRNAs could be translated into small regulatory peptides. It is believed that these circRNAs include an open reading frame (ORF), an internal ribosome entry site (IRES), and N6-methyladenosine (to induce the initiation of protein translation). Circ-ZNF609, circ-FBXW7, and circSHPRH are among the well-known protein-coding circRNAs. These small regulatory peptides have various biological functions including regulating cellular function, tumor inhibition, and translation of circRNAs; however the function of many of these small proteins remains unclear [20,21]. (6) It is believed that some circRNAs such as circ-Amotl1 and circ-Foxo3 function as a scaffold to support a protein complex by facilitating protein localization and function [20].

CircRNAs seem to play important roles in various biological

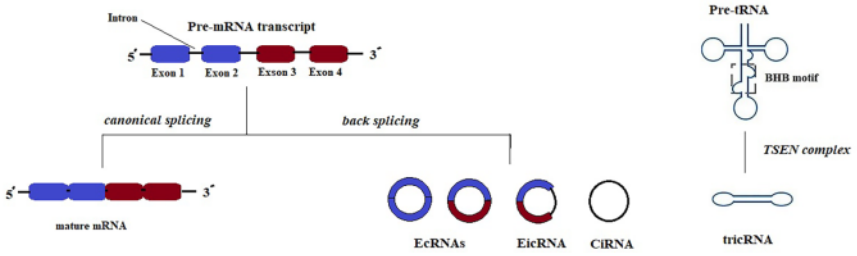

Fig. 1. The biogenesis of circRNAs. EcRNAs: Exonic circRNAs; EIciRNAs: Exonic-intronic circRNAs; ciRNAs: pre-mRNA intronic circRNAs; tricRNAs: pre-tRNA intronic circRNAs; TSEN: tRNA splicing endonuclease.

functions including development, growth, proliferation, and differentiation [22,23]. In addition, in various pathological diseases such as cancers, the aberrant expression of circRNAs has been reported [24]. Recently, it has been identified that some of the circRNAs are involved in osteogenesis [9] and their upregulation or downregulation may be contributed to pathological disorders such as osteoporosis [25]. In the following section, the functional role of circRNAs in osteogenesis has been discussed.

3. CircRNAs during osteogenesis

3.1. Differential expression

Initial studies have demonstrated that circRNAs sequences are highly conserved and their expression is specific to the developmental stage or tissue/organ. Therefore, they could be used as a biomarker or therapeutic target. CircRNAs are involved in organ/tissue development such as neural development and embryonic development. They are also involved in the development of a variety of human diseases [26–28]. Many cellular activities have been thought to be mediated by circRNAs as they are able to regulate gene expression at transcriptional, post-transcriptional, and translational levels [10]. It is also shown that circRNAs could regulate the osteogenic differentiation of stem cells during bone development [9,25]. Some studies have explored the differential expression of circRNAs during osteogenic differentiation of stem cells.

Studies have revealed that circRNAs are differentially expressed during osteogenic differentiation of non-human stem cells [29,30]. Afterward, researchers explored the differential expression of circRNAs during osteogenesis. Kang et al have differentiated AD-MSCs into osteo-like cells and evaluated the differential expression of circRNAs in AD-MSCs and differentiated cells. Screening by microarray analysis has indicated that 290 circRNAs are differentially expressed in which 171 circRNAs were upregulated and 119 circRNAs were downregulated. The qPCR analysis confirmed at least 8 differentially expressed circRNAs with up to 2-fold changes between AD-MSCs and differentiated cells including has_circ_0001421, has_circ_0006618, has_circ_0002890and has_circ_0005752 (upregulating circRNAs) and hsa_circ_0034528, hsa_circ_0002938, hsa_circ_0001766, and hsa_circ_0003251 (downregulating circRNAs). In silico functional analysis indicated that these circRNAs mediate their osteogenesis functions through PI3K-Akt, MAPK, and forkhead box O (FOXO) signaling pathways [31]. A study by Li and coworkers showed the differential expression of 650 circRNAs (333 upregulated and 317 downregulated) between stem cells from apical papilla (SCAPs) and differentiated osteo-like cells. Among them, circNFATC1 had an important role in the osteogenic differentiation of stem cells by sponging miR-4483 [31]. Recently, Huang et al have drawn a circRNA landscape during the adipogenic and osteogenic differentiation of human MSCs. They revealed the differential expression of 1166 circRNAs with lineage-specific expression patterns [32].

3.2. The pathways

Several intercellular signaling pathways are involved in osteogenesis. Some of the circRNAs with inducing or inhibitory effects could regulate these pathways. Table 1 shows circRNAs, the pathways, and the mechanisms that experimentally have been proven to regulate the osteogenesis process.

3.2.1. Wnt/β-catenin signaling pathway

The wnt/β-catenin signaling pathway has a crucial role in bone homeostasis. In this pathway, the binding of the ligands to the Frizzled and LRP5/6 receptors results in the activation and translocation of β-catenin from the cytoplasm to the nucleus, where it binds to DNA-binding proteins and regulate the expression of genes related to osteogenesis [33]. In vivo, the wnt/β-catenin signaling pathway has been reported to repair bone defects [34], enhance bone healing capacity [35], stimulate the expression of bone morphogenetic proteins (BMPs), and promote the expression of alkaline phosphatase (ALP) and Runx2 [36,37].

Glycogen synthase kinase 3β (GSK-3β) negatively regulates the wnt/β-catenin signaling pathway by phosphorylation and degradation of β-catenin [38]. miR-199 negatively regulates the GSK-3β expression and therefore, induces osteogenesis [39]. CircIGSF11 has been shown to sponge miR-199 and inhibit the osteogenic differentiation of stem cells [40]. Dkk1 is an inhibitor of wnt/β-catenin pathway receptors. miR-107, miR-335, and miR-210 downregulate Dkk1 and promote the

Table 1
CircRNAs that regulate osteogenesis.

CircRNA	Study	Pathway	Mechanism	Reference
Hsa_circRNA_33287	In vitro osteogenic differentiation of MSMSCs	Wnt/β-catenin pathway	Hsa_circRNA_33287/miR-214-3p/Runx3 axis	[44]
CircRNA124534	In vitro and in vivo osteoblastic differentiation of PDLSCs	Wnt/β-catenin pathway	CircRNA124534/miR-496/β-Catenin	[82]
Circ_0067680	In vitro osteogenic differentiation of BM-MSCs	Wnt/β-catenin pathway	Circ_0067680/miR-4429/CTNNB1 axis	[45]
Circ_FBLN1	In vitro osteogenic differentiation of BM-MSCs	Wnt/β-catenin pathway	Circ_FBLN1/let-7i-5p/FZD4 axis	[94]
CiRS-7	In vitro osteoblastic differentiation of PDLSCs	P38-MAPK pathway	CiRS-7/miR-7/GDF5 axis	[47]
Hsa_circ_0066523	In vitro osteogenic differentiation of BM-MSCs	PI3/AKT signaling pathway	Hsa_circ_0066523/KDM5B/PTEN	[61]
Mm9_circ_009056	In vitro osteogenesis of MC3T3 cells	TGF-β signaling pathway	Mm9_circ_009056/miR-22-3p/BMP7	[55]
CircRNA FAT1	In vitro osteoblastic differentiation of PDLSCs	TGF-β signaling pathway	CircRNA FAT1/miR-4781-3p/SMAD5 axis	[53]
Circ_0000020	In vitro osteogenic differentiation of primary BM-MSCs	TGF-β signaling pathway	Circ_0000020/miR-142-5p/BMP2 axis	[54]
CircRFWD2	In vitro osteogenic differentiation of DPSCs	TGF-β signaling pathway	circRFWD2/miR-6817-5p/BMPR2 axis	[56]
CircSIPA1L1	In vitro osteogenic differentiation of DPSCs	TGF-β signaling pathway	CircSIPA1L1/miR-617/Smad3 axis	[85]
Circ_AFF4	In vitro osteogenic differentiation of BM-MSCs	TGF-β signaling pathway	Circ_AFF4/miR-135a-5p/FNDC5 axis	[95]
Circ_0138959	In vitro osteogenic differentiation of PDLCs	NF-κB signaling pathway	Circ_0138959/miR-495-3p/TRAF6 axis	[59]
Circ_0087960	In vitro osteogenic differentiation of PDLSCs	SKP2 ubiquitination-related pathway	Circ_0087960/ KDM5B/SKP2 axis	[62]
Circ_0062582	In vitro osteogenic differentiation of BM-MSCs	CBFB-related osteogenic transcription factor stability pathway	Circ_0062582/miR-145/CBFB axis	[63]
Circ_0019693	In vitro osteogenic differentiation of BM-MSCs	PCP4-related calcium deposition pathway	Circ_0019693/miR-942-5p/ PCP4	[64]

MSMSCs: Maxillary sinus membrane stem cells; PDLCs: Periodontal ligament cells; PDLSCs: Periodontal ligament stem cells; BM-MSCs: Bone marrow-derived mesenchymal stem cells; CBFB: Core-binding factor subunit β; DPSCs: Dental pulp stem cells; CTNNB1: catenin beta 1; PTEN: phosphatase and tensin homolog; KDM5B: lysine demethylase 5B; FNDC5: Fibronectin Type III Domain Containing 5).

osteogenesis process by inducing the wnt/β-catenin signaling pathway [41,42]. CircRNA436 has been shown to negatively regulate miR-107 and miR-335 [43]. Moreover, hsa_circ_0127781 also negatively regulates miR-335 and miR-210 [40]. As these miRNAs are considered as positive inducers of the wnt/β-catenin signaling pathway, circRNA436 and hsa_circ_0127781 could inhibit the osteogenesis process. The expression of these circRNAs decreases during osteogenic differentiation of stem cells. Runt-related transcription factor 3 (Runx3) which is regulated by Runx2, promotes osteogenesis by inducing wnt/β-catenin pathway. Hsa_circRNA_33287 has been reported to target Runx3 and inhibit the osteogenesis process through Hsa_circRNA_33287/miR-214-3p/Runx3 axis [44]. Circ_0067680 has been also shown to induce osteogenic differentiation of BM-MSCs through miR-4429/CTNNB1 axis. CTNNB1 (catenin beta 1) is a component of wnt/β-catenin pathway [45]. Fig. 2 shows the regulation of wnt/β-catenin pathway by circRNAs during osteogenesis.

3.2.2. Mitogen-activated protein kinases (MAPKs) signaling pathway

Mitogen-activated protein kinases (MAPKs) signaling pathway plays a crucial role in osteogenic differentiation of stem cells and proliferation of osteoblasts [46]. GDF5 is one of the mediators of the MAPKs pathway which is closely related to p38-MAPK and its silencing has been reported to suppress the osteogenic process. Circular RNA sponge for miR-7 (ciRS-7) which is antisense to the cerebellar degeneration-related protein 1 transcript (CDR1as) regulates osteogenesis by sponging miR-7. While GDF5 mRNA is a target for miR-7, ciRS-7 could promote osteogenesis by suppressing miR-7 [47]. Several other circRNAs have been reported to regulate osteogenesis through the MAPKs pathway. Bioinformatic analysis has shown that circRNA BANP and circRNA ITCH target miRNA34a and miRNA146a respectively and regulate osteogenesis [48]. However, the exact mechanisms of these circRNAs remain unclear and need to be elucidated.

3.2.3. TGF-β signaling pathway signaling pathway

The TGF-β signaling pathway directs the expression of osteogenic-related genes including *runx2* and *ostrix*. Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily mediating their biological roles through Smads-dependent and non-Smads-dependent pathways. BMP ligands bind to BMP receptors (BMPRI/BMPR2 complex) and activate R-Smad1/5/8 by phosphorylation to form a complex with co-Smad4. The activated complex translocates into the nucleus to regulate the expression of several genes including *runx2* and *ostrix*. It has been shown that miRNAs play an important role in the regulation of the TGF-β pathway. For example, miR-195 and miR-17 suppress smad-5 and smad-7 respectively, and negatively regulate the pathway [49,50]. On contrary, miR-20a is involved in the induction of the BMP pathway and upregulates the expression of osteogenic-related genes [51]. Some circRNAs could suppress miRNA activity and regulate the TGF-β pathway. Bioinformatic analysis that circ19142 and circ5846 suppress miR-7067 and induce the TGF-β pathway to regulate osteoblast differentiation [52]. A study by Ye et al demonstrated that circFAT1 positively regulates the osteoblastic differentiation of periodontal ligament stem cells (PDLSCs) through miR-4781-3p/SMAD5 axis [53]. Zhou and coworkers have reported that circ_0000020 induces the osteogenic differentiation of primary BM-MSCs by sponging miR-142-5p and subsequent upregulating of BMP2 [54]. CircRNA_33287 which suppresses miR-214-3p could induce osteogenesis through the TGF-β pathway [44]. In addition, mm9_circ_009056 could induce osteogenesis through the miR-22-39/BMP7 axis [55]. CircRFWD2 also induced the osteogenic differentiation of dental pulp stem cells (DPSCs) through miR-6817-5p/BMPR2 axis [56]. Therefore, circRNAs could modulate osteogenesis by regulating TGF-β signaling pathway components.

3.2.4. NF-κB signaling pathway

Toll-like receptors (TLRs) are transmembrane glycoproteins that are known as immune system components. However, they have also a regulatory role in inducing or inhibiting osteogenesis. TLR4 is considered as a potent enhancer of bone resorption [57]. On contrary, TLR3 could induce osteogenic differentiation of osteoblast possibly through the NF-κB signaling pathway [58]. Bioinformatic analysis has shown that circRNA3140 could induce the osteogenic differentiation of stem cells.

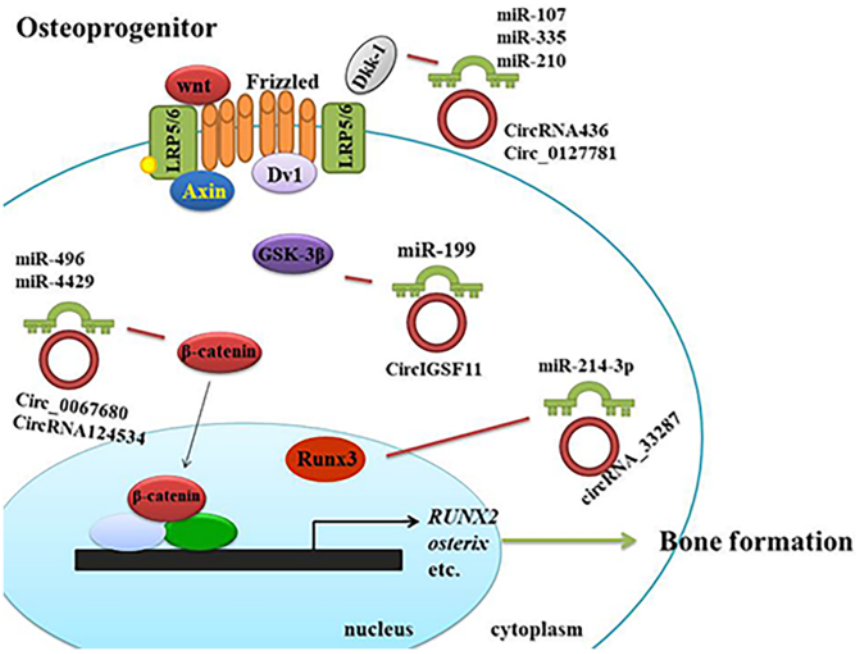

Fig. 2. Regulation of wnt/β-catenin pathway by circRNAs during osteogenesis.

The circRNA3140 expression is closely associated with TLRs expression and the NF-κB signaling pathway [43]. Recently, Deng et al have shown that circ_0138959 regulates the osteogenic differentiation of periodontal ligament cells (PDLCs) by upregulating TNF Receptor Associated Factor 6 (TRAF6). They indicated that circ_0138959 directly inhibits miR-495-3p which is an inhibitor of TRAF6 [59].

3.2.5. PI3/AKT signaling pathway

PI3/AKT signaling pathway is a multifunctional pathway involving in many cellular functions. The abnormal upregulation of the pathway has been shown in many types of cancers [60]. Moreover, the down-regulation of the pathway may change the cell fate. The pathway also has a role in osteogenesis. Therefore, suppressing the pathway may disrupt the osteogenesis process. A study by Xin et al indicated that hsa_circ_0066523 which is derived from forkhead box P1 (FOXP1) upregulated the PI3/AKT signaling pathway by suppressing phosphatase and tensin homolog (PTEN) through epigenetically activating lysine demethylase 5B (KDM5B). PTEN is a direct inhibitor of the PI3/AKT signaling pathway [61].

3.2.6. Other pathways

S-phase kinase associated protein-2 (SKP2) is a protein that induces the ubiquitination and subsequent degradation of RUNX2. Circ_0087960 has been shown to induce osteogenesis of periodontal ligament stem cells (PDLSCs) by suppressing the function of SKP2 [62]. Another study showed that circ_0062582 positively regulates the osteogenic differentiation of BM-MSCs by stabilizing osteogenic transcription factors through the miR-145/CBFB axis [63]. Circ_0019693 has been shown to induce osteogenesis-coupled angiogenesis in BM-MSCs by sponging miR-942-5p and subsequent overexpression of Purkinje cell protein 4 (PCP4) [64] which is involved in calcium deposition possibly through c-Jun NH2-terminal kinase (JNK) signaling pathway [65].

4. CircRNA as an inducer in osteogenic differentiation

Bone is considered as a self-healing organ in which bone remodeling restores bone structure and function and maintains bone integrity over time. Bone remodeling affords the healing of small bone defects less than 8 mm in size [66]. However, major injuries and defects that are caused by trauma, infections, tumors, or congenital disorders could not be healed by bone remodeling and in such cases, painful surgical interventions and bone substitutes are used [67]. Standard bone substitutes that are used in clinics include autograft or allograft ilium, tibia, and fibula. While the use of autograft bones ensures no immune rejection, its clinical usage is limited due to the side effects at the donor site as well as the shortage of graft sources. In addition, there is a risk of disease transmission when allograft bone is used. As a result, an alternative to bone substitute is required to be used in clinics. Bone engineered grafts fabricated by the tissue engineering approaches may fulfill the demands for bone substitutes [68].

Functional tissue/organ regeneration is the ultimate goal of tissue regeneration. In this regard, tissue engineering approaches are employed polymers, stem cells, and growth factors/inducers to generate a construct to be used as a bone substitute (Fig. 3) [69]. Mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs), by differentiating into osto-like cells (osteoblasts and odontoblasts), have been widely used in bone tissue engineering [70,71]. Osteogenesis includes the formation of osteoblasts and odontoblasts, which depend on a different microenvironment in vivo [72,73]. Many inducers have been used to differentiate stem cells into osteo-like cells. Small molecules including ascorbic acid, β-glycerophosphate (βGP), and dexamethasone (DEX) have been widely used during the osteogenesis of stem cells [74]. Bone tissue-specific genes and miRNAs have been also used [75–77]. Due to their role in osteogenesis, researchers have used circRNAs to induce osteogenic differentiation of stem cells.

A study by Han et al showed the upregulation of circ_0076690 during the osteoblastic differentiation of BM-MSCs. They demonstrated that the

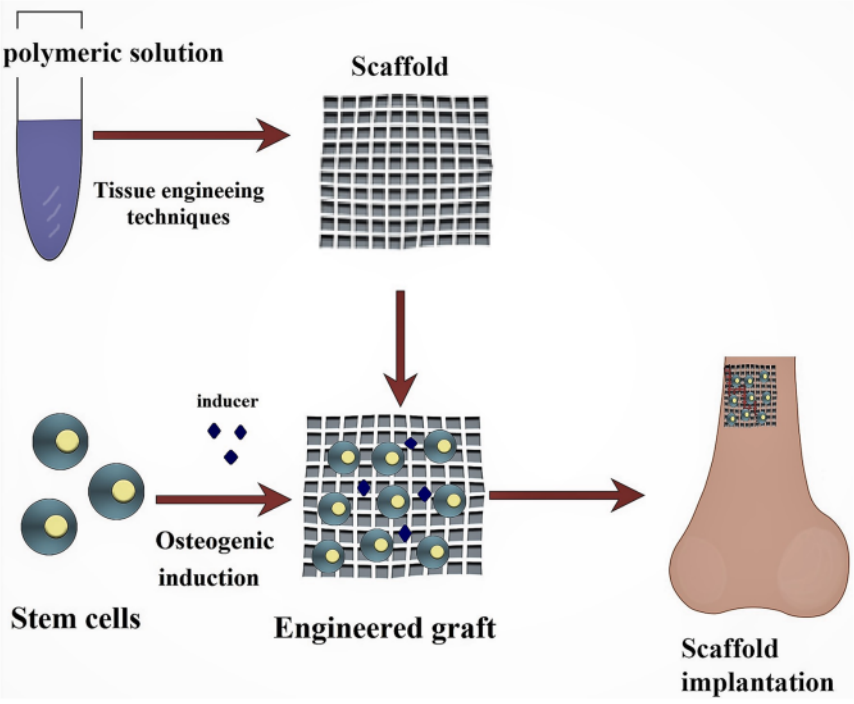

Fig. 3. Bone tissue engineering. Stem cells, polymeric scaffolds, and growth factors/inducers are the main components of bone tissue engineering.

overexpression of circ_0076690 could increase the expression of RUNX2 by targeting miR-152. They also found that miR-152 is a negative regulator of osteogenesis and directly inhibits the expression of RUNX2 [78]. In another study, Ji and colleagues overexpressed to induce osteogenic differentiation of BM-MSCs. They found that circ_0006215 induces osteogenic differentiation through miR-942-5p/RUNX2 axis [79]. Jiang et al also showed that the overexpression of circ-0007292 promotes the osteogenic differentiation of posterior longitudinal ligament cells [80]. Similar results were observed when circRNA-23525 was overexpressed in AD-MSCs [81]. Ji and coworkers have evaluated the circRNAs that have been overexpressed during osteogenic differentiation of MSCs. They found that circRNA124534 overexpressed during osteoblastic differentiation. The overexpression of circRNA124534 in human periodontal ligament stem cells (hPDLSCs) induced osteogenesis in vitro and in vivo. They showed that circRNA124534 acts through the miR-496/β-Catenin pathway to induce osteogenesis [82]. In another study, Ji et al also showed that overexpressing circ_0026827 in hDPSCs induces heterotopic bone formation in BALB/c homozygous nude mice [83]. Another study also indicated that upregulating circFOXP1 in AD-MSCs could induce heterotopic bone formation in vivo [84]. Similar results were observed when CDR1as was used to induce heterotopic bone formation in a critical-sized mouse calvarial defect model [47]. Huang and colleagues found that circRFWD2 overexpression in DPSCs induced osteoblastic differentiation by activating TGF-β signaling pathway [56]. Another study by Ge et al showed that circSIPA1L1 could also induce osteoblastic differentiation of DPSCs through TGF-β signaling pathway [85]. The results of these studies indicated the role of circRNAs as a potential inducer to promote osteogenic differentiation of stem cells to be used in bone tissue engineering.

5. CircRNA dysregulation during bone-related diseases

The dysregulation of circRNAs has been reported in bone-related diseases such as osteoporosis, osteoarthritis, and osteosarcoma [86]. Osteoporosis is a prevalent bone disease that is characterized by low bone density and high bone fragility. The dysregulation of signaling pathways (i.e., Wnt and RANKL-RANK pathways) is involved in the pathogenesis of the disease. Some miRNAs such as miR-506-3p and miR-7223-3p inhibit bone resorption. It is shown that circUBAP2 and circRNA AFF4 suppress miR-506-3p and miR-7223-3p, and induce bone resorption [86]. Another study showed that circ_0011269 sponges miR-122 and thereby promoting osteoporosis [87]. Osteoarthritis is characterized by chronic inflammation, cartilage degradation, and bone thickening. Inflammatory agents play a crucial role in the pathogenesis of osteoarthritis, particularly by inducing oxidative stress. It is reported that circRNA.33186 is involved in the early stage of osteoarthritis by sponging miR-127-5p and increasing the expression of matrix metalloproteinase-13 (MMP-13) [88]. CircRFWD2 and circINO80 induce the expression of IL-1β and influence osteoarthritis [86]. Other circRNAs such as CircCDH13 and CircRNA-UBE2G1 have been shown to be dysregulated in osteoarthritis [89,90]. However, the exact mechanism of these circRNAs needs to be elucidated. Osteosarcoma is a common bone cancer which is mostly happened in the epiphysis of long bones. CircUBAP2 has been shown to induce osteosarcoma by sponging miR-641 and increasing the expression of YAP1 [91]. In addition, aberrant expression of circAGFG1 and circular RNA PRKAR1B may also correlate with the progression of osteosarcoma [92,93]. As circRNAs play a role in the progression of bone-related diseases, targeting these specific circRNAs might be a promising non-invasive treatment option for such diseases.

6. Concluding remarks

CircRNAs were first considered as a by-product of aberrant splicing of primary transcripts. By the growing evidence, now, it is well known that they play a crucial role in the regulation of gene expression in eukaryotes. Therefore, they are believed to be involved in many cellular functions such as development, proliferation, differentiation, and migration. Later studies indicate the role of circRNAs in osteogenesis which could provide a biomarker for diagnosis of bone-related diseases. CircRNAs have been shown to be able to regulate many cellular pathways including wnt/β-catenin, MAPKs, TGF-β, NF-κB, PI3/AKT, and other pathways to induce osteogenesis in vitro and in vivo. Therefore, circRNAs might be a suitable inducer to promote osteogenic differentiation of stem cells in bone tissue engineering approaches. While differential expression analyses have shown that hundreds of circRNAs might be involved during osteogenesis, only some of them have been used as an inducer for osteogenic differentiation of stem cells. In addition, further differential expression analyses with larger sample sizes are required to confirm the expression level of circRNAs during osteogenesis. CircRNA dysregulation has been reported in bone-related diseases such as osteoporosis and osteonecrosis. This shows that circRNAs could also be a target for targeted therapy of such diseases. However, the studies are relatively new, and more researches are needed to explore the exact mechanisms of circRNAs during osteogenesis and map the specific interactions between circRNAs-miRNAs-mRNAs during osteogenesis. In addition, more studies are required to evaluate the potential of specific circRNAs as a biomarker or therapeutic target for osteo-related diseases.

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Ahmed Hjazi: Conceptualization. **Bayu Indra Sukmana:** Writing – original draft. **Sally Saad Ali:** Writing – original draft. **Hashem O. Alsaab:** Writing – original draft. **Jitendra Gupta:** Writing – original draft. **Muhammad Ikram Ullah:** Writing – review & editing. **Rosario Mireya Romero-Parra:** Supervision. **Ahmed H.R. Alawadi:** Writing – review & editing. **Adeeb Abdulally Abdulhussien Alazbjee:** Project administration. **Yasser Fakri Mustafa:** Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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