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REVIEW ARTICLE

Role of circular RNAs in determining the fate of mesenchymal stem cells

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Abstract

Ribonucleic acid (RNA) has been well-understood for its linear form for many years. With advances in high-throughput sequencing, there is an increasing focus on circular RNAs (circRNAs) recently. Although they were previously regarded as splicing error by-products, research has shown that they play a pivotal role in many cellular processes, one of which is the control of stem cell differentiation and fate. On the other hand, decades of research have demonstrated the promising therapeutic potential of mesenchymal stem cells (MSCs). To this end, there is a growing body of research on the role of circRNAs in the determination of the fate of MSCs. This review critically examines the current evidence and consolidates key findings from studies that explore the involvement of circRNAs in the regulation of MSC differentiation.

Keywords: circular RNA, mesenchymal stem cell, regulation, differentiation

INTRODUCTION

Ribonucleic acid (RNA) is an important polymeric molecule that is found in all biological cells. Traditionally, the understanding of RNA has been limited to its linear form, which plays a pivotal role in protein synthesis. However, with advances in technologies, many other types of RNA have been discovered in the past few decades, including non-coding RNAs (ncRNAs), which represent 98% of the human RNAs.[1] The ncRNAs vary in their lengths ranging from 20 to a few hundred nucleotides.[2] In general, ncRNA can be divided into two broad classes, i.e. regulatory ncRNAs and housekeeping ncRNAs. Some examples of the former include microRNA (miRNA), long-non-coding RNA (lncRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), enhancer RNA (eRNA), and circular RNA (circRNA). On the other hand, ribosomal RNA (rRNA), small nuclear RNA (snRNA), transfer RNA (tRNA), and small nucleolar RNA (snoRNA) belong to the latter.[3]

The discovery of circRNA as viroids in RNA viruses can be dated back to the mid-1970s. CircRNAs were previously thought to be by-products of splicing error. Nevertheless, as scientists learn more about their structural characteristics and functions, circRNAs begin to gain much attention and become an interesting area of research. They have been demonstrated to function as miRNA sponges,[4] interact with RNA binding proteins (RBPs),[5] and play a key role in the regulation of transcription and gene expression.[6] Although they are generally considered ncRNAs, studies have found that circRNAs can be translated into proteins.[7] In addition, dysfunction of circRNAs may lead to a wide range of medical conditions and they have been demonstrated to play a part in the pathogenesis of diseases such as malignancies,[8] cardiovascular diseases,[9] neurodegenerative diseases[10] etc., suggesting that circRNAs may be promising therapeutic targets and biomarkers.

Mesenchymal stem cells (MSCs) are a type of non-haemopoietic multipotent stem cells that have been widely studied in the past few decades, especially in the context of stem cell-based therapy, tissue engineering,

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and regenerative medicine. These cells can be obtained from many sites in the body such as the bone marrow, adipose tissue, placenta, cord blood, and dental pulp. Other names that are commonly used to describe this population of cells include mesenchymal stromal cells, marrow stromal cells, mesenchymal progenitor cells, and multipotent stromal cells. MSCs can be readily harvested from various tissues and cultured in the laboratory. Research has shown that they exhibit immunomodulatory and anti-inflammatory effects and that they are capable of secreting an array of beneficial growth factors and cytokines.[11]

As circRNAs play a pivotal role in gene expression and regulation of protein functions, cellular events like cell proliferation and differentiation, as well as cell death are regulated by circRNAs. Studies have reported that differentiation and other functions of MSCs are likewise, regulated by circRNAs. Processes in MSCs that are associated with circRNAs include adipogenic-,[12] osteogenic- and chondrogenic differentiation.[13] This review gives an overview of the biogenesis, expression and functions of circRNAs in general. It critically examines the current evidence concerning the role of cricRNAs in the regulation of MSC differentiation and consolidates the key findings.

1. Circular RNA (circRNA)

Circular RNAs are a type of ncRNA that is transcribed from deoxyribonucleic acid (DNA) with or without being translated to proteins. Several types of circRNA have been described in the published literature and they have been found to play a role in gene expression regulation. This section discusses the biogenesis of circRNAs, the various types of circRNA, as well as the functions of circRNA in general.

1.1 Biogenesis and expression of circRNA

To understand the biogenesis and types of circRNA, it is crucial to understand the basic principles of RNA processing. Just like the linear RNAs, circRNAs are synthesized from premature mRNA (pre-mRNA), a primary transcript that is derived from DNA. Most circRNAs are produced through a process referred to as backsplicing. Backsplicing can result in three main types of circRNA, i.e. circRNA consists of exons (exonic circRNA), introns (intronic circRNA) or both exons and introns (exon-intron circRNA).[14] The biogenesis of different types of circRNAs is summarised in Figure 1. As the circRNAs form close loops without the usual 5' and 3' ends, research has shown that they are more stable than linear RNAs derived from the common host gene, with a median half-life that is longer than their linear RNA counterparts.[15]

In mammals, research has revealed that the brain has an abundance of circRNAs.[16] Subcellular analysis of circRNA localisation in the brain revealed that circRNAs are mainly found in the cytoplasm but nuclear localisation has also been demonstrated to a lesser extent.[17] There is also a considerable population of circRNAs circulating in the blood,[18] owing to their stability and resistance to degradation. Many circRNAs have also been identified in different adult and foetal tissues such as heart, lung, kidney, colon, stomach and glands in a tissue-specific manner. Some circRNAs have a stronger abundance in foetal than adult tissues, suggesting their roles in human tissue development.[19] Due to their cell- and tissue-specificity, circRNAs are potential biomarkers in many diseases. For example, many cancer-specific circRNAs have been identified[20] and they may be helpful in disease diagnosis and monitoring and may act as therapeutic targets.

1.2 Functions of circRNA

Unlike the linear RNAs that play a part in protein synthesis, most of the circRNAs are non-protein coding. However, they play a major role in miRNA sponging, RNA-binding protein sponging and gene regulation. Research has shown that circRNAs are involved in cell proliferation, differentiation, cell death such as apoptosis and metastasis.[21]

1.2.1 Role of circRNA in miRNA sponging

MicroRNAs (miRNAs) were first described by Lee *et al*. in 1993[22] and later coined in 2001 by Ruvkun.[23] Many miRNAs have since been discovered. As their name implies, miRNAs belong to a group of small RNAs that are non-coding and naturally occurring, which consist of about 21- 25 nucleotides in length. One of the main functions of miRNA is the downregulation of gene expression by means of translational repression and mRNA degradation. However, they have also been found to upregulate gene expression by responding to different cell types and conditions when distinct cofactors are present.[24]

Post-transcriptionally, studies have shown that miRNAs contribute to the regulation of gene expression in several important cellular events

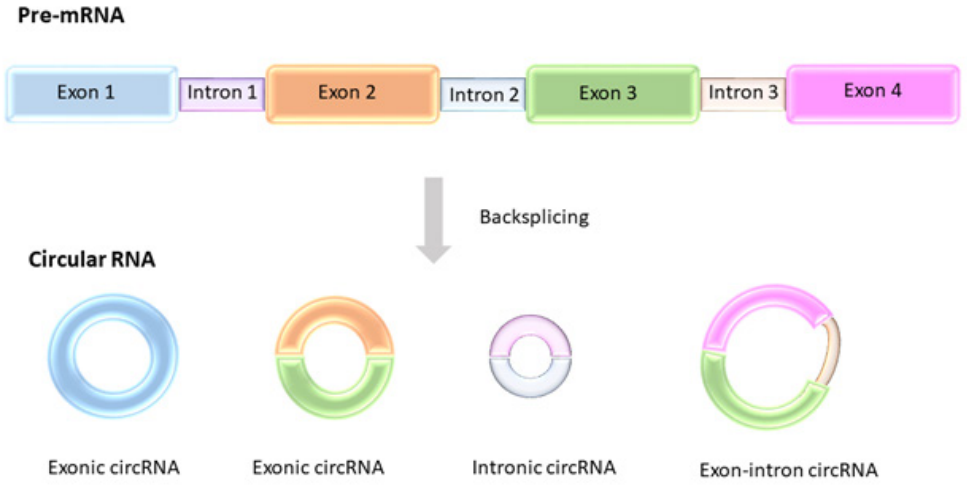

FIG. 1: Biogenesis and types of circRNA. Backsplicing results in circular RNAs consisting of exons, introns or a combination of exons and introns.

like differentiation, migration, proliferation and apoptosis.[25] On the other hand, many circRNAs have been demonstrated to regulate miRNA functions by acting as cytoplasmic miRNA sponges that inhibit the latter's activity.[26] The naturally occurring circRNA sponge for miR-7 (ciRS-7), was first discovered by Hansen *et al*. in human and mouse brains.[4] This circRNA has over 70 miRNA target sites and is capable of exerting strong suppression on miR-7 activity. As co-expression of miR-7 and ciRS-7 was observed, especially in the hippocampal and neocortical neurons, it was suggested that a substantial level of endogenous interaction between the cirRNA and miRNA existed.

1.2.2 Role of circRNA in RNA-binding protein sponging

RNA-binding proteins (RBPs) are a class of proteins that bind RNA molecules and play a role in the post-transcriptional regulation of gene expression. They can interact with proteins and different types of RNAs such as mRNA, tRNA, ncRNA and are important in the formation of ribonucleoprotein (RNP) complexes.[27] Some events associated with RBPs include modulation of miRNA processing and decay, regulation of splicing, alternative polyadenylation, mRNA transport, stability, localisation and translation of RNA.[28,29]

The interaction between circRNAs and RBPs is regarded as an important aspect of circRNA function. CircRNAs act as inhibitors which have an influence on gene expression or cellular functions. Therefore, circRNAs also act as sponges for RBPs. CircRNAs regulate the availability of RBPs in the cell and have an influence on the post-transcriptional fate of the mRNAs that interact with RBPs.[30] In one study, the circRNA CircPABPN1 has been found to suppress the binding of an RBP (known as HuR) to its target mRNA (PABPN1 mRNA) in HeLa cells. The suppression of HuR- PABPN1 mRNA binding led to a reduced level of PABPN1, suggesting that PABPN1 translation was influenced by the suppression of HuR activity by CircPABPN1. However, the levels of HuR or PABPN1 mRNA were not affected by the interaction between CircPABPN1 and HuR.[31]

1.2.3 Other functions of circRNAs

CircRNA have also been found to act as protein decoys and protein scaffolds. CircRNA acting as a protein decoy is illustrated in the ability of circ-Amolt1 to bind cMyc in the nucleus. By doing so, circAmotl1 retains and stabilises cMyc in the nucleus, which leads to upregulation of its target genes, and subsequently an increase in cell proliferation and a reduction in apoptosis.[32] On the other hand, circRNA acting as a protein scaffold and aids in the assembly of protein complexes is shown in the interaction between cir-foxo3, cyclin-dependent kinase 2 (CDK2) and CDK inhibitor p21, which leads to suppression of cell cycle progression. However, when circ-foxo3 is downregulated, CDK2 is released from p21, leading to phosphorylation of cyclin E and cyclin A by CDK2, and subsequent cell cycle progression.[33]

It is noteworthy that although circRNAs were

initially thought to be non-coding, studies have found that some circRNAs can be translated. Circ-ZNF609 has been shown to be translated and contribute to the regulation of myoblast proliferation,[34] whereas circular AKT3 RNA encodes a tumour suppressor protein that inhibits tumourigenicity in glioblastoma.[35] On the other hand, research has shown that the biogenesis of circRNA has an impact on the splicing machinery as the production of circRNA competes with the production of linear RNA from pre-mRNA. When there is an increase in canonical splicing, a reduction of circRNA levels is observed.[36] This competition between the linear and circRNAs may have an effect on gene expression as the mRNAs may lack the exons which have been used for circRNA production.

2. Mesenchymal stem cells (MSCs) and their properties

MSCs are a population of multipotent stem cells found in many locations in the body whereas the bone marrow and adipose tissue remain an abundant source of MSCs. Like many other types of stem cells, MSCs exhibit the ability to self-renew and differentiate, particularly into cells of mesodermal origin such as chondrocytes, adipocytes and osteoblasts. However, earlier studies have demonstrated the ability of MSCs to transdifferentiate into cells with endodermal and neuroectodermal characteristics.[37]

MSCs are known for possessing immune tolerant properties and for being immune-privileged. The interactions between MSCs and the immune system are complex and are beyond the scope of this review. MSCs regulate the activities of immune cells by cell contact or via their secretory products. MSCs have also been found to exert immunomodulatory and immunosuppressive effects in both the innate and adaptive immune systems (reviewed by Li *et al*.).[38] In addition, these cells have been shown to create a favourable regenerative environment at the site of injury and home to sites of inflammatory where they secrete paracrine factors and soluble substances such as growth factors, chemokines and cytokines.[39,40]

There are many reports on the therapeutic potential of MSCs. MSCs are harvested from many different sites of the body and are readily cultured in the laboratory. Other reasons why they are popular among researchers and clinicians include the multipotent nature, immunomodulatory and immunosuppressive effects of MSCs, making MSCs potential therapeutic candidates for regenerative medicine,[41] cancer[42] and autoimmune diseases.[43]

3. CircRNAs regulate MSC differentiation

Differentiation of MSCs involves two major steps. The first step is lineage commitment, during which MSCs become committed to progenitors that are lineage specific. The second step is maturation, during which the committed progenitors develop into specific cell types. Many signalling pathways have been implicated in the differentiation of MSCs, such as the platelet-derived growth factor (PDGF) pathway, fibroblast growth factor (FGF) signalling, transforming growth factor-beta (TGF-β) pathway, mitogen-activated protein kinase (MPK) signalling, Akt pathway, Wingless and Int-1 (Wnt) and insulin signalling pathway.[44] On the other hand, many chemical and physical factors can influence the differentiation of MSCs and the fate of MSCs.[45] More recently, there is a growing body of research that explores the role of circRNAs in the control of MSC differentiation. Evidence that circRNAs regulate the fate of MSCs from various sources is increasing. This section examines circRNAs that play a part in MSC differentiation with a focus on the underlying mechanisms involved.

3.1.1 Adipogenesis

One of the cell types that MSCs differentiate into is the adipocytes. In adipogenesis, the MSCs are first committed to the adipogenic lineage without morphological changes. This is followed by maturation during which the preadipocytes accumulate lipids and become functional mature adipocytes that are insulin responsive.[46] Several circRNAs have been associated with MSC adipogenic differentiation. In one study, the circRNA FOXP1 was reported to inhibit mRNA and sustain MSC identity. To exclude source-related biases, both bone marrow- and umbilical blood-derived MSCs were used to explore circRNAs that were associated with the definition or fate decision of MSCs. CircFOXP1 was demonstrated to be the strongest regulated circRNA in MSCs. When MSCs were induced to differentiate into adipocytes, osteocytes and chondrocytes, there was a reduction in the expression levels of circFOXP1, suggesting that circFOXP1 is a marker for undifferentiated MSCs. On the other hand, the capacity of MSC to differentiate into adipocytes and osteocytes was diminished by silencing circFOXP1, suggesting that circFOXP1 may act as a transcriptional

regulator in the sustenance of MSC identity and stemness.[47]

In another study, Chen *et al*. reported that circRNA CDR1as was shown to promote the adipogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) in steroid-induced osteonecrosis of the femoral head (SONFH).[48] As many as 820 circRNAs were expressed differentially in SONFH-BMSCs whereas circRNA CDR1as was of particular importance in adipogenic/osteogenic differentiation disorder. CDR1as was observed to promote adipogenic differentiation and inhibit osteogenic differentiation in SONFH-BMSCs. However, when CDR1as was knocked down, the reverse occurred, with enhancement in osteogenic differentiation and attenuation of adipogenic differentiation. CDR1as targeted WNT5B (an mRNA) by acting as a sponge for the miRNA miR-7-5p. The CDR1as–miR-7p–WNT5B axis was hypothesised to contribute to the pathogenesis of osteogenic/adipogenic differentiation disorder. The suppression of osteogenic differentiation was believed to occur due to sponging of miR-7-5p, which led to increased expression of WNT5B, which then inhibited β-catenin, favouring adipogenic differentiation over osteogenic differentiation. This, in turn, enhanced the accumulation of adipose tissue and hindered bone repair in SONFH.[48]

On the contrary, human circular RNA H19 (hsa_circH19) has been shown to inhibit adipogenesis in adipose-derived stem cells (hADSCs), which are mesenchymal-like cells. Silencing hsa_circH19 led to an increase in adipogenic gene expression and lipid droplet formation. When hsa_circ H19 was depleted, sterol-regulatory element-binding proteins (SREBP1) translocated from the cytoplasm to the nucleus in the presence of polypyrimidine tract-binding protein 1 (PTBP1). It was concluded that enhanced adipogenic differentiation of hADSCs occurred when hsa-circH19 was knocked down via PTBP1 targeting. Hence, hsa_circH19 is believed to play a part in the lipid metabolism of adipose tissue from those with metabolic syndrome.[49]

In summary, cirRNAs influence adipogenesis by sustaining MSC identity and sponging miRNAs. Research has shown that circFOXP1 sustained the stemness of MSCs and silencing circFOXP1 resulted in diminished differentiation capacity of MSCs. CircRNA CDR1 favoured adipogenic differentiation over osteogenic differentiation and plays a role in osteogenic/adipogenic differentiation disorder in patients with SONFH while silencing hsa_circH19 enhanced adipogenesis in patients with metabolic syndrome. The effects of circRNA on MSC adipogenic differentiation in different studies appear to occur via different miRNAs, target proteins or signalling pathways.

3.1.2 Osteogenesis

The first MSC differentiation identified by Friedenstein *et al*. in earlier studies was osteogenic differentiation.[50] The ability of MSCs to differentiate into osteocytes is not surprising as the bone marrow is a rich source of MSCs. The osteogenic differentiation capacity of MSCs makes them one of the most hopeful stem cells in bone tissue engineering, as well as regenerative medicine. Osteogenic differentiation of MSCs often involves several growth factors, pathways and genes that are important for osteogenesis. During the early phase of *in vitro* osteogenic differentiation, the expression of alkaline phosphatase (ALP), conversion of type I procollagen to collagen I and deposition of the extracellular matrix that is collagenous in nature are observed. This is followed by other markers such as osteonectin, osteopontin, osteocalcin and bone sialoprotein. The final marker for osteogenic differentiation is the appearance of hydroxyapatite deposits.[51]

Zhang *et al*. reported that the circRNA-vgll3 plays a role in osteogenic differentiation of adipose-derived mesenchymal stem cells (ADSCs) from Sprague Dawley rats via a circRNA-vgll3/miR-326-5p/integrin α5 (Itga5) pathway.[52] Normally, Itga5 acts as a promoter of ADSC osteogenic differentiation, while the activity of Itga5 is suppressed by miR-326-5p as the latter inhibits Itga5 translation. The effect of circRNA-vgll3 on osteogenic differentiation was mediated via its direct sequestration of miR-326-5p. When this occurred, mir-326-5p no longer exerted an inhibitory effect on Itga5, thus increasing osteogenic differentiation. Furthermore, circRNA-vgll3 was shown to enhance the formation of new bone and bone mineral density, as well as to increase trabeculae number and bone/ tissue volume. The study concluded that circRNA-vgll3 engineered ADSCs may be beneficial in bone regenerative medicine.[52]

Another circRNA, circRNA-23535 was reported to be upregulated during adipose-derived mesenchymal stem cell (ADSC)

osteoblastic differentiation. circRNA-23535 mediates its effects via the circRNA-23535/ miR-30a-3p/Runt-related transcription factor 2 (RUNX2) axis. When circRNA-23535 was upregulated, miR-30a-3p was downregulated and the expression of RUNX2 increased as a result. RUNX2 is a known transcription factor that plays a role in the osteogenic differentiation of MSCs. Therefore, it is believed that circRNA-23535 regulated osteogenic differentiation of ADSCs via regulation of RUNX2 by targeting miR-30a-3p. On the other hand, knocking down of circRNA-23534 demonstrated the opposite effects, with increased expression of miR-30a-3p and decreased expression of RUNX2.[53]

Cherubini *et al*. showed that when circFOXP1 was silenced in MSCs, there was a reduction in osteogenic differentiation of these cells with a significant decrease in matrix mineralisation.[47] In addition, knocking down circFOXP1 demonstrated impaired bone repair capacity of MSCs in an atrophic non-union rat femur model when compared to the control group. The control group showed normal union, normal bone rigidity. On the other hand, the group with silenced circFOXP1 showed an absence of bone bridge at the site of osteotomy, clearly detectable fracture line, suboptimal bone healing, histologically evident non-union that was filled with fibrous tissue and without bone ridges.

Human dental pulp stromal cells (hDPSCs) are MSC-like populations that share many characteristics of bone marrow-derived MSCs including their proliferative and clonogenic properties.[54] In one study, the circRNA circAKT3 was reported to play a role in hDPSC osteogenesis. The effects of circAKT3 were mediated via the circAKT3/miR-206/ CX43 axis. CircAKT3 directly inhibited miR-206 by acting as a sponge and positively regulated CX43 in osteogenesis. When circAKT3 was knocked down *in vivo*, mineralised nodule formation and osteogenic protein expression were attenuated.[55] Estrogen receptor beta (ERβ) deficiency was found to alter the expression of several circRNAs and influence osteogenic differentiation of rat bone marrow MSCs (rBMSCs). Inhibition of osteogenesis was demonstrated to be via the circRNAs 2:27713879|27755789 and 2:240822115|240867796, whereas miR-328-5p was identified to be the target miRNA.[56]

An array of circRNAs like circRNA-vgll3, circRNA 23535, circFOXP1, circ AKT3, circRNAs 2:27713879|27755789 and 2:240822115|240867796 play a role in regulating MSC osteogenic differentiation. Different target miRNAs (miR-30a-3p, miR-326-5p, miR-206, miR-328-5p) and proteins (Itga5, RUNX2, CX43) have been identified for the effects of circRNAs on MSC osteogenic differentiation, suggesting that the signalling may be distinct. One possible explanation for these findings is the use of different differentiation conditions and MSCs from different sources. However, other factors may also be involved and warrant further exploration.

3.1.3 Chondrogenesis

MSCs are promising candidates in cartilage regenerative medicine as they have the potential to differentiate into chondrocytes under certain culture conditions. In a study by Della Bella *et al*. several circRNAs were demonstrated to be differentially expressed during early osteo- and chondrogenic differentiation of MSCs.[13] For osteogenic differentiation, a total of 21 circRNAs were upregulated and 21 circRNAs, downregulated whereas as many as 130 circRNAs were upregulated and 97 circRNAs were downregulated during MSC chondrogenic differentiation. These circRNAs were derived mainly from four sets of common genes, namely, FKBP5, ZEB1, FADS2 and SMYD3. The first three were upregulated whereas the last gene was downregulated. However, the role of these circRNAs awaits further exploration.[13] The role of circRNAs in MSC differentiation is summarised in Table 1.

3.1.4 Role of circRNAs in regulating MSCs in other tissues

Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) have been reported to play a role in the repair of damaged endometrium by enhancing angiogenesis. When co-cultured with damaged endometrial stromal cells (ESCs), WJ-MSCs have been shown to enhance ESC proliferation, with an increase in vascular angiogenesis marker expression via circ6401. MiR-29b-1-5p was identified as the target miRNA whereas the target protein was Rap1B, a protein that plays a crucial role in the VEGF signalling pathway.[57] In another study, a total of 226 circRNAs were differentially expressed when human umbilical cord-derived MSCs differentiated into cardiomyocyte-like cells with three of them identified as key circRNAs, namely, circRNA_08441, circRNA_05432 and circRNA_01536.[58]

CONCLUSIONS

Research in the area of circRNAs has become increasingly important and popular due to their emerging role in regulating many key cellular events, whereas MSCs have been well-known for their promising clinical use and therapeutic potential. Not only have circRNAs been associated with many disease processes, circRNAs have also been demonstrated to influence the differentiation and fate of stem cells. Particularly, abundant pieces of evidence point to the role of circRNAs in regulating MSCs differentiation and destiny. CircRNAs have been demonstrated to sustain the stemness and differentiation capacity of MSC, and play a part in MSC adipogenic, chondrogenic and osteogenic differentiation. A number of circRNAs and the underlying pathways through which circRNAs exert their effects on MSC differentiation have been identified. It is unsure why there exist differences in the underlying pathways. One possible explanation is the use of different culture conditions and different sources of MSCs. Other factors may have contributed to the underlying mechanisms and are worth further exploration.

Interestingly, some circRNAs promote

TABLE 1: Summary of the role of circular RNAs in the regulation of MSC differentiation and fate

Circular RNA/ Targeted miRNA/ Targeted protein	Source of MSCs	Differentiation/ process involved	Key findings	Reference
circRNA FOXP1	Human bone marrow- and umbilical blood-derived MSCs	Adipogenic, osteogenic and chondrogenic differentiation	• A marker for undifferentiated MSCs • CircFOXP1 expression reduced when MSCs differentiated into adipocytes, chondrocytes and osteoblasts, • Silencing circFOXP1 reduced differentiation potential of MSCs • Knocking down circFOXP1 showed impaired bone repair capacity of MSCs in rat model	Cherubini *et al*., 2019 [47]
circRNA CDR1as **Target miRNA: miR-7-5p** **Target protein: WNT5B**	Human bone marrow-derived mesenchymal stem cells (hBMSCs)	Adipogenic and osteogenic differentiation	• Favoured adipogenic differentiation of BMSCs over osteogenic differentiation in steroid-induced osteonecrosis of the femoral head (SONFH) • Played a role in the pathogenesis of osteogenic/adipogenic differentiation disorder • Targeted WNT5B by acting as a sponge for miR-7-5p	Chen *et al*., 2020 [48]
hsa_circH19	Human adipose-derived stem cells (hADSCs)	Adipogenic differentiation	• Silencing hsa_circH19 led to enhanced adipogenic differentiation of hADSCs • May play a role in lipid metabolism in metabolic syndrome.	Zhu *et al*., 2020 [49]
circRNA-vgll3 **Target miRNA: miR-326-5p** **Target protein: integrin α5 (Itga5)**	Adipose-derived mesenchymal stem cells (ADSCs) from Sprague Dawley rats	Osteogenic differentiation	• Promoted osteogenic differentiation via circRNA-vgll3/miR-326-5p/integrin α5 (Itga5) pathway • Enhanced formation of new bone and bone mineral density and increased trabeculae number and bone/ tissue volume	Zhang *et al*., 2021 [52]
circRNA-23535 **Target miRNA: miR-30a-3p** **Target protein: RUNX2**	Adipose-derived mesenchymal stem cells (ADSCs)	Osteogenic differentiation	• Promoted osteogenic differentiation via circRNA-23535/miR-30a-3p/Runt-related transcription factor 2 (RUNX2) axis.	Guo *et al*., 2020 [53]

Circular RNA/ Targeted miRNA/ Targeted protein	Source of MSCs	Differentiation/process involved	Key findings	Reference
circAKT3 **Target miRNA: miR-206** **Target protein: CX43**	Human dental pulp stromal cells (hDPSCs)	Osteogenic differentiation	• Promoted osteogenic differentiation via circAKT3/miR-206/ CX43 axis. • Knockdown of circAKT3 decreased in vivo hDPSC bone mineralization.	Zhang *et al*., 2020 [55]
Several upregulated and downregulated circular RNAs	Human bone marrow-derived mesenchymal stem cells (hBMSCs)	Osteogenic and chondrogenic differentiation	• 21 upregulated and 21 downregulated circRNAs identified for osteogenic differentiation • 130 upregulated and 97 downregulated circRNAs identified for chondrogenic differentiation. • CircRNA derived from 4 common sets of genes i.e. FKBP5, ZEB1, FADS2 and SMYD3	Della Bella *et al*., 2020 [13]
circRNAs 2:27713879|27755789 & 2:240822115|240867796 **Target miRNA: miR-328-5p**	Rat bone marrow MSCs (rBMSCs)	Osteogenic differentiation	• Deficiency in ERβ altered expression of circRNAs 2:27713879|27755789 and 2:240822115|240867796 and influenced osteogenic differentiation of rBMSCs by targeting miR-328-5p	Li *et al*., 2017 [56]
Circ6401 **Target miRNA: miR-29b-1-5p** **Target protein: RAP1B**	Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs)	Endometrial stromal cell proliferation and angiogenesis	• Enhanced endometrial stromal cell proliferation and angiogenesis via circ6401/mir-29b-1-5p/ RAP1B axis.	Shi *et al*., 2020 [56]
circRNA_08441 circRNA_05432 circRNA_01536	Human umbilical cord-derived MSCs	Differentiation into cardiomyocyte-like cells	• Differential expression of 226 cicRNAs when hUMC-MSCs differentiated into cardiomyocte-like cells with identification of three key circRNAs (circRNA_08441, circRNA_05432 and circRNA_01536)	Ruan *et al*., 2019 [58]

differentiation while others inhibit differentiation, whereas the effects of circRNAs are observed across MSCs from different sources. Taken together, circRNAs are promising therapeutic targets in MSC-based tissue engineering and regenerative medicine. As many studies have focussed on screening of upregulated and downregulated circRNAs in MSC differentiation and on circRNAs function as a miRNA sponge, future studies should investigate other mechanisms through which circRNAs influence the fate of MSCs. Investigation on the binding sites of circRNAs on the target miRNA may also be beneficial, as this helps researchers to better comprehend the functional roles of circRNAs and manipulate MSC differentiation into the desired tissues. Lastly, other than differentiation, circRNAs may play a role in other MSC-related processes that determine the fate of MSCs or other cells, which are also worth exploring in future research.

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REFERENCES

1. ENCODE Project Consortium: An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489: 57-74.
2. Neelabh N, Gautam A. Noncoding RNA. In: Vonk J, Shackelford T (eds) Encyclopedia of Animal Cognition and Behavior. Springer, Cham; 2018. https://doi.org/10.1007/978-3-319-47829-6_192-1

3. Zhang P, Wu W, Chen Q, Chen M. Non-Coding RNAs and their Integrated Networks. J Integr Bioinform. 2019; 16(3):20190027.
4. Hansen TB, Jensen TI, Clausen BH, *et al*. Natural RNA circles function as efficient microRNA sponges. Nature. 2013; 495(7441): 384-8.
5. Zang J, Lu D, Xu A. The interaction of circRNAs and RNA binding proteins: An important part of circRNA maintenance and function. J Neurosci Res. 2020; 98(1): 87-97.
6. Huang S, Yang B, Chen BJ, *et al*. The emerging role of circular RNAs in transcriptome regulation. Genomics. 2017; 109(5-6): 401-7.
7. Yang Y, Fan X, Mao M, *et al*. Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res. 2017; 27: 626-41.
8. Mei M, Wang Y, Li Z, Zhang M. Role of circular RNA in haematological malignancies. Oncol Lett. 2019; 18(5): 4385-92.
9. Prestes PR, Maier MC, Woods BA, Charchar FJ. A Guide to the short, long and circular RNAs in hypertension and cardiovascular disease. Int J Mol Sci. 2020; 21(10): 3666.
10. Akhter R. Circular RNA and Alzheimer's disease. Adv Exp Med Biol. 2018; 1087: 239-43.
11. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen Med. 2019; 4: 22.
12. Wang F, Li X, Li Z, Wang S, Fan J. Functions of Circular RNAs in Regulating Adipogenesis of Mesenchymal Stem Cells. Stem Cells Int. 2020; 2020: 3763069.
13. Della Bella E, Menzel U, Basoli V, Tourbier C, Alini M, Stoddart MJ. Differential Regulation of circRNA, miRNA, and piRNA during early osteogenic and chondrogenic differentiation of human mesenchymal stromal cells. Cells. 2020; 9(2): 398.
14. Greene J, Baird AM, Brady L, *et al*. Circular RNAs: biogenesis, function and role in human diseases. Front Mol Biosci. 2017; 4: 38.
15. Enuka Y, Lauriola M, Feldman ME, Sas-Chen A, Ulitsky I, Yarden Y. Circular RNAs are long-lived and display only minimal early alterations in response to a growth factor. Nucleic Acids Res. 2016; 44(3): 1370-83.
16. Rybak-Wolf A, Stottmeister C, Glažar P, *et al*. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. Mol Cell. 2015; 58(5): 870-85.
17. Zaghlool A, Ameur A, Wu C, *et al*. Expression profiling and in situ screening of circular RNAs in human tissues. Sci Rep. 2018; 8(1): 16953.
18. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of Circular RNAs as a new class of putative biomarkers in human blood. PLoS One. 2015; 10(10): e0141214.
19. Xu T, Wu J, Han P, Zhao Z, Song X. Circular RNA expression profiles and features in human tissues: a study using RNA-seq data. BMC Genomics. 2017;18 (Suppl 6):680.
20. Xia S, Feng J, Chen K, Ma Y, *et al*. CSCD: a database for cancer-specific circular RNAs. Nucleic Acids Res. 2018; 46(D1): D925-D929.
21. Haque S, Harries LW. Circular RNAs (circRNAs) in health and disease. Genes (Basel). 2017; 8(12): 353.
22. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75(5): 843-54.
23. Ruvkun G. Molecular biology. Glimpses of a tiny RNA world. Science. 2001; 294(5543): 797-9.
24. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. Int J Genomics. 2014; 2014: 970607.
25. Panda AC. Circular RNAs act as miRNA sponges. Adv Exp Med Biol. 2018; 1087: 67-79.
26. Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: Identification, biogenesis and function. Biochim Biophys Acta. 2016; 1859(1): 163-8.
27. Gerstberger, S.; Hafner, M.; Tuschl, T. A census of human RNA-binding proteins. Nat. Rev. Genet. 2014; 15: 829-45.
28. Kang D, Lee Y, Lee JS. RNA-binding proteins in cancer: functional and therapeutic perspectives. Cancers (Basel). 2020;12(9):2699.
29. Oliveira C, Faoro H, Alves LR, Goldenberg S. RNA-binding proteins and their role in the regulation of gene expression in Trypanosoma cruzi and Saccharomyces cerevisiae. Genet Mol Biol. 2017;40(1):22-30.
30. Hentze MW, Preiss T. Circular RNAs: splicing's enigma variations. EMBO J 2013; 32:923-5.
31. Abdelmohsen K, Panda AC, Munk R, *et al*. Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1. RNA Biol. 2017;14(3):361-9.
32. Yang Q, Du WW, Wu N, *et al*. A circular RNA promotes tumorigenesis by inducing c-Myc nuclear translocation. Cell Death Differ. 2017; 24:1609–20.
33. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res. 2016;44(6):2846-58.
34. 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: 22-37.
35. Xia X, Li XX, Li FY, *et al*. A novel tumor suppressor protein encoded by circular AKT3 RNA inhibits glioblastoma tumorigenicity by competing with active phosphoinositide-dependent Kinase-1. Mol Cancer. 2019; 18: 131.
36. Ashwal-Fluss R, Meyer M, Pamudurti NR, *et al*. circRNA biogenesis competes with pre-mRNA splicing. Mol Cell. 2014; 56(1): 55-66.
37. Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J. 2004; 18(9): 980-2.
38. Li H, Shen S, Fu H, *et al*. Immunomodulatory functions of mesenchymal stem cells in tissue engineering. Stem Cells Int. 2019; 2019: 9671206.
39. Murphy MB, Moncivais K, Caplan AI. Mesenchymal

stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013; 45: e54.
40. da Silva ML, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev. 2009; 20: 419-27.
41. Patel DM, Shah J, Srivastava AS. Therapeutic potential of mesenchymal stem cells in regenerative medicine. Stem Cells Int. 2013; 2013:496218.
42. Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic potential of mesenchymal stem cells for cancer therapy. Front Bioeng Biotechnol. 2020; 8: 43.
43. Kahmini FR, Shahgaldi S. Therapeutic potential of mesenchymal stem cell-derived extracellular vesicles as novel cell-free therapy for treatment of autoimmune disorders. Exp Mol Pathol. 2020; 118: 104566.
44. Saidova AA, Vorobjev IA. Lineage Commitment, signaling pathways, and the cytoskeleton systems in mesenchymal stem cells. Tissue Eng Part B Rev. 2020; 26(1): 13-25.
45. Chen Q, Shou P, Zheng C, *et al*. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ. 2016; 23(7): 1128-39.
46. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006; 7(12): 885-96.
47. Cherubini A, Barilani M, Rossi RL, *et al*. FOXP1 circular RNA sustains mesenchymal stem cell identity via microRNA inhibition. Nucleic Acids Res. 2019; 47(10): 5325-40.
48. 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.
49. 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.
50. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968; 6: 230-47.
51. Hanna H, Mir LM, Andre FM. In vitro osteoblastic differentiation of mesenchymal stem cells generates cell layers with distinct properties. Stem Cell Res Ther. 2018; 9(1): 203.
52. Zhang D, Ni N, Wang Y, *et al*. CircRNA-vgll3 promotes osteogenic differentiation of adipose-derived mesenchymal stem cells via modulating miRNA-dependent integrin α5 expression. Cell Death Differ. 2021; 28(1): 283-302.
53. 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. 2020.
54. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000; 97: 13625-30.
55. Zhang B, Huo S, Cen X, Pan X, Huang X, Zhao Z. circAKT3 positively regulates osteogenic differentiation of human dental pulp stromal cells via miR-206/CX43 axis. Stem Cell Res Ther. 2020; 11(1): 531.
56. Li X, Peng B, Zhu X, Wang P, *et al*. Changes in related circular RNAs following ERβ knockdown and the relationship to rBMSC osteogenesis. Biochem Biophys Res Commun. 2017; 493(1): 100-7.
57. Shi Q, Sun B, Wang D, *et al*. Circ6401, a novel circular RNA, is implicated in repair of the damaged endometrium by Wharton's jelly-derived mesenchymal stem cells through regulation of the miR-29b-1-5p/RAP1B axis. Stem Cell Res Ther. 2020; 11(1): 520.
58. 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): 16412-23.