



# Hypoxia-Inducible Non-coding RNAs in Mesenchymal Stem Cell Fate and Regeneration

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Mesenchymal stem cells (MSCs) can differentiate into multiple cell lines, which makes them an important source of cells for tissue engineering applications. They are defined by the capability to renew themselves and maintain pluripotency. This ability is modulated by the balance between complex cues from cellular microenvironment. Self-renewal and differentiation abilities are regulated by particular microenvironmental signals. Oxygen is considered to be an important part of cell microenvironment, which not only acts as a metabolic substrate but also a signal molecule. It has been proved that MSCs are hypoxic in the physiological environment. Signals from MSCs' microenvironment or niche which means the anatomical location of the MSCs, maintain the final properties of MSCs. Physiological conditions like oxygen tension are deemed to be a significant part of the mesenchymal stem cell niche, and have been proved to be involved in modulating embryonic and adult MSCs. Non-coding RNAs (ncRNAs), which play a key role in cell signal transduction, transcription and translation of genes, have been widely concerned as epigenetic regulators in a great deal of tissues. With the rapid development of bioinformatics analysis tools and high-throughput RNA sequencing technology, more and more evidences show that ncRNAs play a key role in tissue regeneration. It shows potential as a biomarker of MSC differentiation. In this paper, we reviewed the physiological correlation of hypoxia as a unique environmental parameter which is conducive to MSC expansion and maintenance, discussed the correlation of tissue engineering, and summarized the influence of hypoxia related ncRNAs on MSCs' fate and regeneration. This review will provide reference for future research of MSCs' regeneration.

**Keywords:** hypoxia, mesenchymal stem cell, non-coding RNA, differentiation, niche

## INTRODUCTION

### Hypoxia Microenvironment in Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent a subset of adult stem cells derived from the mesoderm lineage. The identification standard established by the International Society for Cell Therapy (ISCT) is to prove the positive expression characteristics of CD73, D90 and CD105, and lack of expression of CD14, CD34, CD45, and human leukocyte antigen-DR (HLA-DR) (1, 2). As non-hematopoietic

progenitor cells, MSCs can be obtained from a variety of human tissues, which include bone marrow, adipose tissue, skin and dental pulp, etc (3, 4). Multiple tissue cells derived from the mesoderm and neuroectoderm can be differentiated by MSCs, such as bone marrow stromal cells (BMSCs), fibroblasts, osteoblasts, chondrocytes, adipocytes, and vascular endothelial cells, even neurons and glial cells of the nervous system (5–7).

MSCs *in vivo* help maintain and regulate the homeostasis of related organs, and the modification or transplantation of MSCs seems to be the preferred treatment method in a variety of pathological processes (8). Specifically, stem cells are life-long reservoirs of several cell types, which have the characteristics of fighting inflammation and other diseases. Hence, MSCs have been used as the most practical stem cells in the treatment of different systemic diseases (9).

Stem cells and their precursors exist in the stem cell niche (10), which is a particular cell microenvironment. Several recent literatures suggest in the stem cell niche, hypoxia environment plays a key role for keeping the phenotype of precursor cells and undifferentiated stem cells (11). Hypoxia helps stem cells to stay in a quiescent status and has the necessary self-renewal rate. The response of stem cells to hypoxia is one of the main mechanisms for adapting to changes in the external and internal environment. It has been observed for decades that stem cells can respond immediately to hypoxia (12). Recent findings have shown that hypoxia not only induces the proliferation of several stem cells *in vitro*, but also participates in stem cells differentiation and survive (13, 14).

Inflammation and malignant tumors all present hypoxic environment, and bone reconstruction under physiological condition is also strictly controlled by signaling pathways related to hypoxia. The physiological bone reconstruction might be caused by the hypoxic environment of the bone cell niche (15). Studies in mice show that the lowest oxygen tension is 1.3 kPa (10 mmHg; tissues lower than this level are commonly known as hypoxic tissues) (16, 17). Mammalian adaptation to hypoxia at the cellular and systemic levels is mainly provided by the transcription regulator hypoxia inducible factor (HIF). HIFs are heterodimeric transcription factors, including a  $\alpha$  subunit (HIF-1 $\alpha$ , HIF-2 $\alpha$ , HIF-3 $\alpha$ ) and a composition  $\beta$  subunit (HIF- $\beta$ ) which is vital in inducing the response of cells to hypoxia (18, 19). HIF1- $\alpha$  and HIF2- $\alpha$  are similar in structure (20). The stability of these molecules is dominated by the oxygen availability through the iron-dependent enzyme prolyl hydroxylase (PHDs) after transcription (21).

Different oxygen pressure and hypoxia exposure time may play important and different roles in cell development. Effects of hypoxia on the influences of MSCs of literature were shown in **Table 1**. Transcription factors and cytokines obviously affect these processes. Studies have proved that factors which regulate the behavior of stem cells and progenitor cells have an important relation with HIFs. The HIFs act as a molecular framework for hypoxia to control differentiation and survival of cells (43). Hypoxia is by no means a purely pathological state. Hypoxia often occurs under various physiological conditions, especially when rapid tissue growth lags behind the development of blood supply. First, it

is related to embryogenesis under 1-5% oxygen content. However, this situation is not harmful to normal embryonic development; moreover, it seems to be an indispensable and completely “selected” one in the evolutionary process of mammalian embryo formation. This may indicate that under hypoxic conditions, all developing tissues have regular relationships (10).

In some aspect, hypoxia is thought to be the better environment for the maintenance and function of various stem cell types (such as embryos, hematopoiesis, mesenchymal and neurons). It promotes stem cells to remain at rest at the necessary rate of self-renewal. There is a certain regulatory mechanism to maintain hypoxic state in stem cell niche. This microenvironment basically affects the proliferation and differentiation ability of stem cells and protects cells against the toxicity and mutagenicity of oxygen free radicals. The key element of these mechanisms is HIF, a complex of hypoxia inducible transcription factors (10).

Under hypoxia condition, HIF complexes can induce Notch signaling by attaching to Notch receptors (44). Notch signal plays a key role in the determination of cell survival. Notch signal induced by HIF has been proved to be related to the determination of stem cell fate. In relative studies, hypoxia and HIFs have been shown to be involved in osteogenesis, cartilage formation, angiogenesis, heart formation and the formation of neural crest (34, 45–47). With the introduction of new technologies into this field, the development of single cell RNA sequencing, transcriptome sequencing and material technology, recent researches have vividly described some epigenetic changes, including hydroxy methylation, histone modification, DNA methylation, organ like and three-dimensional structure formation in early embryonic development, induced pluripotent stem cells (iPSCs) and somatic reprogramming. The interaction between hypoxia and epigenetics has potential application in regenerative medicine (48). Hypoxia can affect the level of epigenetic modification of cells. The coexistence of transcription factors suggests that hypoxia may have a relation with epigenetics in cell reprogramming regulation (48).

It is undeniable that HIF signaling and epigenetics are involved in the events that lead to stem cell proliferation and differentiation. The review may help deepen the understanding of tissue regeneration microenvironment.

## Non-coding RNAs

Non-coding RNAs (ncRNAs) are molecules that have no or very low ability to code protein. In fact, although most human genes can be transcribed into RNAs, some of the RNAs cannot be translated into proteins (49). Recently, with the progress of multidisciplinary methods and high-throughput technologies, we begin to know more about ncRNAs and its' signaling network. Generally speaking, ncRNAs are found to be involved in a variety of biological processes, regulating physiological and developmental processes and even diseases.

Based on different regulatory effects, ncRNAs are generally divided into two categories. Housekeeping ncRNAs are widely expressed in cells and mainly modulate the general function of cells. Although regulatory ncRNAs are generally regarded

**TABLE 1** | Effects of Hypoxia on the influences of MSCs.

MSCs types	Oxygen concentration	Influences	References
Human bone marrow-derived	1%	Induce migration and angiogenesis	(22)
Human dental pulp	1%	Enhance the angiogenic potential	(23)
Human bone marrow-derived	5%	Enhance clonogenicity but impair differentiation	(24)
Human bone marrow-derived	2%	Induce osteogenic differentiation	(25)
Human bone marrow-derived	3%	Inhibit osteogenic differentiation and enhances stemness	(26)
Rat bone marrow-derived	1%	Regulate the osteogenesis in a time-dependent manner	(27)
Human dental pulp	1%	Accelerate bone healing	(28)
Human bone marrow-derived	1%	Induction of adipocyte-like phenotype	(29)
Human bone marrow-derived	2%	Enhance proliferation and tissue formation	(30)
Placental-derived	5%	Promote osteogenesis	(31)
Human bone marrow-derived	1%	Reduce proliferation and differentiation	(32)
Human urine/dental pulp/amniotic fluid	5%	Enhance cell proliferation rate, retention of stem cell properties, inhibition of senescence, and increased differentiation ability	(33)
Human bone marrow-derived	1%	Enhance chondrogenesis	(34)
Rat bone marrow-derived	5%	Enhance osteochondrogenesis	(35)
Human dental pulp	5%	Promote mineralization	(36)
Murine bone marrow	1%	Promote the progression of the chondrocyte lineage	(37)
Human apical papilla	1%	Promotes osteogenic and neurogenic lineages	(38)
Human periodontal ligament	2%	Induce osteogenesis and angiogenesis	(39)
Human bone marrow-derived	1%	Increase chemotaxis migration	(40)
Human bone marrow-derived	2%	Reduce osteogenic differentiation	(41)
Human periodontal ligament	2%	Enhance osteogenic differentiation	(42)

as significant RNA molecules, they play a regulatory role in gene expression at the epigenetic, transcription and post-transcriptional levels (50–52).

According to the average size of regulatory ncRNAs, they can be further divided into small ncRNAs containing <200 nt transcripts and long non-coding RNAs (lncRNAs) larger than 200 nt. Most of small RNAs can be divided into small interfering RNAs (siRNAs), microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs). However, a number of variable-length non-coding RNAs may belong to both categories, like enhancer RNAs, circular RNAs (circRNAs) and promoter-related transcripts (53).

MiRNAs are endogenous, single-strand small ncRNAs and consist of 20–25 nucleotides. With a complex enzymatic pathway, they are usually synthesized from larger RNAs transcripts. MiRNAs can be transcribed into pri-miRNAs by RNA polymerase II or III, or processed from ncRNAs, or processed from introns of protein-coding genes (miRtrons) (54). As the largest type of small non-coding RNAs produced by the transcribed hairpin loop structure (55, 56). miRNAs could modulate gene expression in and outside nucleus with different biological mechanisms, and silence gene after transcription (56). As a key participant in the complex interaction between different RNAs species, miRNAs have been widely concerned by researchers in recent years.

lncRNAs refer to transcripts with a length of more than 200 nt and no capabilities to code protein. According to their position relative to genes that code protein, lncRNAs can be divided into

five subclasses (57): (1) promoter-related lncRNAs, (2) enhancer-related lncRNAs, (3) natural antisense transcripts, (4) genome-related (sense) lncRNAs and (5) long intergenic ncRNAs (58). Based on their regulatory effects on DNA sequences, lncRNAs can be subdivided into trans-lncRNAs (trans-acting lncRNAs) and cis-lncRNAs (cis-acting lncRNAs), the former regulating the expression of close-range genes (59). Some ncRNAs can be further processed to produce small ncRNAs, like miRNAs (60).

CircRNAs are endogenous circular RNAs with a covalent closed loop structure. Although it has been discovered for many years, circRNAs has recently received further attention with the development of bioinformatics analysis and deep sequencing (61). CircRNAs are mostly formed by the “reverse splicing” of pre-mRNAs and are mainly found in the cytoplasm or exosomes (62, 63). These circRNAs are more stable than linear RNAs because they have a special form with a covalent ring instead of linear form, which makes it hard for ribonuclease R to degrade them (64). CircRNAs can be divided into four types: ciRNAs, ecircRNAs, tricRNAs, EIciRNA, based on the pattern of biogenesis and the source of the genome. The size range of circRNAs is very wide, from 100 nt to more than 10,000 nt, and most circRNA in mammals or plants are made of hundreds of nucleotides (64, 65). Just like other regulatory non-coding RNAs, circRNAs work in plenty of biological processes, like modulating alternative RNA splicing or transcription (62), acting a competitive role as endogenous RNA (ceRNAs) (66) or miRNA sponges (67).

Thanks to the rapid development of sequencing technology and bioinformatics methods, in the past few decades, different types of ncRNAs have been found, constructing a complicated ncRNAs system. There is ample evidence that ncRNAs acts an important biological role in regulating cellular mechanisms. They may regulate cell behaviors of stem cells in their niche. In addition, complex microenvironmental factors may also induce a variety of ncRNAs to influence the fate of these cells.

## Hypoxia Induced ncRNAs

Under hypoxia, more than 800 target genes related to different signaling pathways such as apoptosis, proliferation, metabolism and angiogenesis were activated. Hypoxia inducible factor-1 (HIF-1) plays an important role in hypoxia microenvironment as a key mediator (68). HIF-1 complex transcriptionally activates ncRNAs in response to hypoxia. Under normal circumstances, PHDs and other proline hydroxylases hydroxylate the subunits of HIF-1/2 $\alpha$ . The von Hippel-Lindau (VHL) protein further recognizes the hydroxylated HIF-1/2 $\alpha$  as a target for subsequent ubiquitination and proteasome degradation. Under hypoxic conditions, HIF-1/2 $\alpha$  cannot be hydroxylated and gradually accumulates, nuclear translocation, and forms dimers with HIF- $\beta$ . It forms a transcription initiation complex with the recruited CBP/p300 and binds to the promoter of the target gene to induce target gene expression. Hypoxia can induce the expression of ncRNAs. ncRNAs including miRNAs, lncRNAs or circRNAs are involved in regulating cell proliferation, cycle and death and other cell behaviors (Figure 1). Some of them can participate in the regulation of HIFs (69). Such as, lncRNA H19 was found highly expressed under hypoxia and may be involved in regulating stem cell behaviors (70). lncRNA HIF-1 $\alpha$ -AS2 facilitates the maintenance of stem cell-like cells in hypoxic niche mesenchymal glioblastoma. The expression of ncRNAs in MSCs was significantly altered under different hypoxia conditions (71). For example, in 0.5% O<sub>2</sub> stimulated BMSCs, 15 common miRNAs (miR-22, miR-29b, miR-214, miR-140-3p, miR-17, miR-24, miR-320a, miR-26a, miR-1233, miR-222, miR-193b, miR-150, let-7f, let-7a, and miR-516a-5p) were significantly changed (72). miR-210 is a major hypoxia-induced miRNA in most cells (73). Dental pulp stem cells (DPSCs) showed stronger proliferation and lower osteogenic/odontogenic differentiation potential under hypoxia. Compared with normoxic group, 60 mRNAs (25 up-regulated, 35 down-regulated), 47 lncRNAs (20 increased, 27 decreased) and 14 miRNAs (7 up-regulated, 7 down-regulated) were differentially expressed in DPSCs in hypoxic group. After a bioinformatics analysis, 7 mRNAs of GRPR, ERO1L, ANPEP, EPHX1, PGD, ANGPT1 and NQO1 and 5 lncRNAs of AF085958, AX750575, UC002CZN.2, RP3-413H6.2 and six twelve leukemia (STL) may be associated with each other. twenty eight mRNAs including GYS1, PRKACB and NQO1 and 13 miRNAs including hsa-miR-3916 and hsa-miR-192-5p may be correlated with each other and play roles (74). There were 102 differentially expressed circRNAs in BMSCs under hypoxia (1% O<sub>2</sub>) pretreatment. Its function may mainly regulate cell development, differentiation and regulation by affecting gene stability. Transcriptional abnormalities are most prominent in the mitogen-activated protein kinase (MAPK) signaling pathway,

which may be the core pathway associated with hypoxia in cells (75).

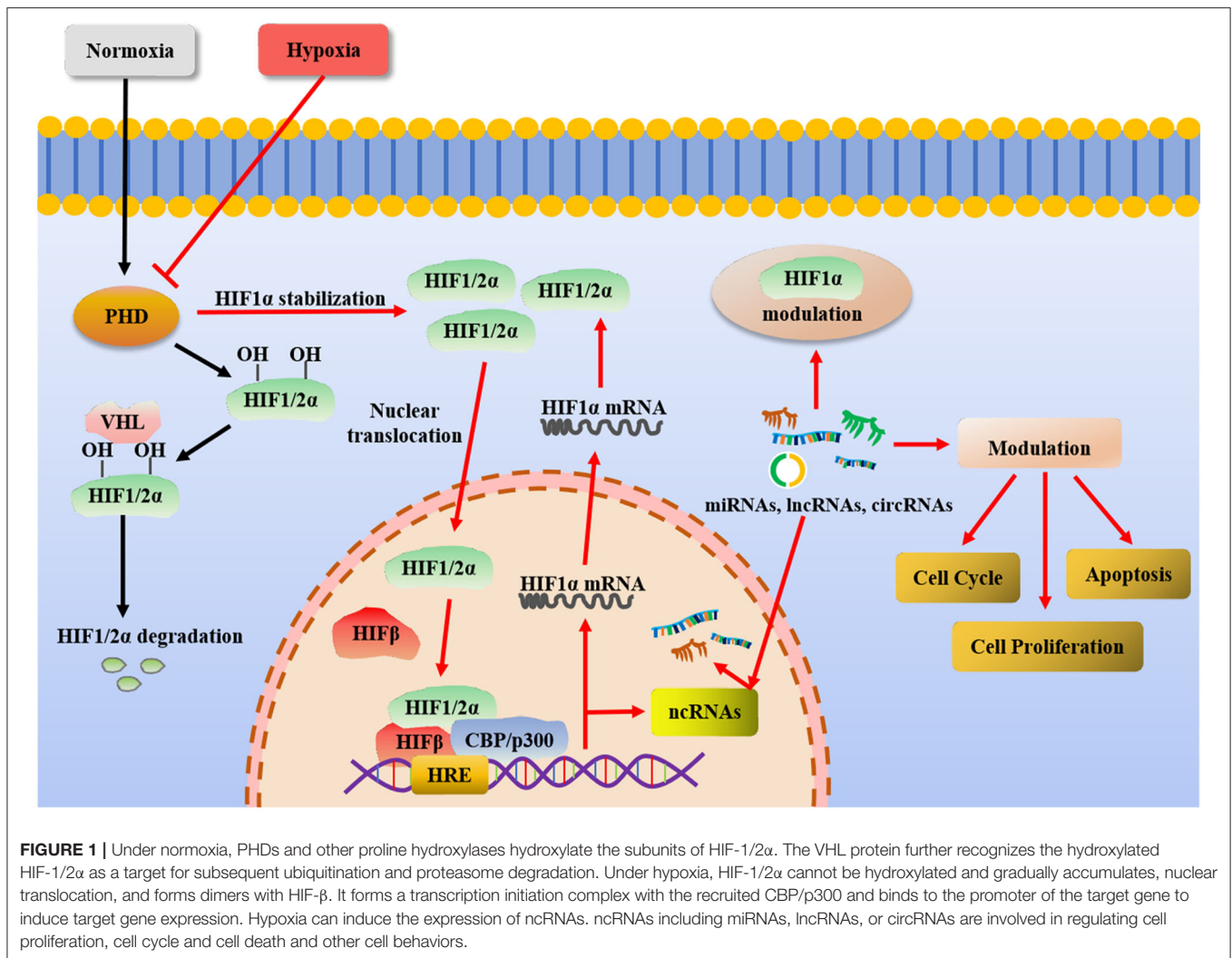
In the previous study of our group, it was found from high-throughput data that a total of, 1046 mRNAs were differentially expressed in periodontal ligament stem cells (PDLSCs) under hypoxia (2% O<sub>2</sub>) and normoxia culture. Compared with normoxia group, 516 mRNAs were up-regulated and 530 were down-regulated under hypoxia. A total of 449 lncRNAs were differentially expressed, of which 406 were increased and 43 decreased in the hypoxia group, and 53 circRNAs were found to be differentially expressed in the hypoxia group, including 17 up-regulated and 36 reduced. In addition, 15 miRNAs were found to be differentially expressed in the hypoxia group, of which 6 were increased and 9 were decreased.

## Hypoxia Induced ncRNAs With a Role in the Fate of MSCs

Studies have shown that BMSCs exhibit greater colony-forming potential in hypoxia (35, 76), proliferate faster (77, 78), and better maintain their undifferentiated properties in hypoxia (26, 30). Hypoxia not only affected the differentiation of BMSCs, but the migration ability and survival rate of MSCs in hypoxia preconditioning group were significantly higher than those in normoxia group (79, 80). In addition, HIF-1 $\alpha$ -mediated SDF-1 expression may be the molecular mechanism of this process (40). In the studies of BMSCs, hypoxia induced senescence of MSCs, possibly through changes in intestinal flora (81). However, most studies have shown that hypoxia can inhibit the senescence of BMSCs and help maintain their characteristics (33, 82, 83). *In vivo*, transplanted MSCs pretreated with hypoxia show higher survival and tissue protection, and microRNA-326 (miR-326) expression in hypoxic MSCs is significantly higher than that in normoxic MSCs (83). Hypoxia pretreatment can simulate physiological conditions, activate the adaptation process of MSCs under physiological conditions, and improve the niche of MSCs, which is conducive to the successful transplantation of MSCs (84). It was found that hypoxic preconditioning of BMSCs (85) and neural stem cells (NSCs) (86) enhances nerve regeneration and promotes neurological recovery after intracerebral hemorrhage.

As exhibited in Table 1, the results showed that the proliferation and differentiation potential of odontogenic MSCs were changed under hypoxia stimulation. However, the results are uncertain due to differences in cell sources and experimental conditions. Hypoxia has been reported to promote the proliferation of odontogenic stem cells (36, 42, 87). Although some scholars believe that the proliferation and survival of odontogenic stem cells are not changed or inhibited under hypoxia (38, 88). The proliferation of PDLSCs was enhanced in 24h under 2% O<sub>2</sub> (39). It was also showed that under different oxygen concentrations (21%, 5%, and 1% O<sub>2</sub>), the proliferation ability of PDLSCs showed a trend of first increasing and then decreasing under lower oxygen concentrations (89). This indicates that for stem cell proliferation, short-term or mild hypoxia may stimulate the adaptive response of cells and





promote proliferation, while severe or long-term hypoxia may lead to cell death.

Most studies have shown that hypoxia-induced ncRNAs participate in the regulation of migration, proliferation, apoptosis, aging and other survival abilities of different MSCs (Table 2). miR-210 supports the survival of stem cells under hypoxia (96). Ischemia-preconditioning (IP) can improve the survival rate of MSCs under hypoxia, and increase the expression of miR-210. Hypoxia-induced lncRNA H19 expression was negatively correlated with cell injury. Further studies suggested that H19 might activate the Wnt/ $\beta$ -catenin and PI3K/AKT pathways by inhibiting the expression of miR-107, and alleviate hypoxia-induced NSC cell damage (97).

It is reported that miR-326 expression is significantly upregulated in hypoxic BMSCs, hypoxic preconditioning upregulates miR-326/PTBP1/PI3K-mediated autophagy (84), lncRNA-P21, HIF-1 $\alpha$ , and CXCR4/7 expressions were increased in hypoxic preconditioning induced BMSCs. The migration and viability of MSCs was promoted by P21 under *in vitro* hypoxic preconditioning (79). DNMT3OS/miR-127-5p/GREM2

may be involved in hypoxic chondrocyte differentiation (95). H19 enhanced the survival rate and angiogenesis potential of BMSCs *in vitro*. It can directly up-regulate VEGFA expression by inhibiting miR-199a-5p as a competitive endogenous RNA (90).

## Hypoxia Induced ncRNAs Participating in the Differentiation of MSCs

Hypoxia stimulation has significant effects on gene expression and is therefore thought to play an important role in early embryonic development, cell reprogramming and differentiation. In general, stem cells show pluripotency and undifferentiated maintenance state under hypoxia (98). Some studies have shown that hypoxia enhances osteogenic/odontogenic differentiation of odontogenic stem cells (28, 88, 99), while others have reported contrary results (100). Moreover, under severe hypoxia (0.01% O<sub>2</sub>) or hypoxia conditions, cell death has been reported to occur (23).

Reduced differentiation of human BMSCs into adipocytes and osteoblasts was found in hypoxic culture (32). However,

**TABLE 2** | Non-coding RNAs involved in regulating the fate and regeneration of MSCs under hypoxia.

ncRNAs	Cell type	Impact of hypoxia on ncRNAs expression	Effects	Mechanism	References
LncRNA-H19/miR-199a-5p	Human MSCs	Up-regulated/down-regulated	Improve survival and angiogenic capacity.	VEGFA/miR-199a-5p axis	(90)
miR-326	Human MSCs	Up-regulated	Delay senescence by upregulating autophagy	PI3K/PTBP1	(84)
LincRNA-p21	Human MSCs	Up-regulated	Promote MSC migration and survival capacity	HIF-1 $\alpha$ /CXCR4 and CXCR7 pathway	(79)
miR-210	Human MSCs	Up-regulated	Increase survival rates	HIF-1 $\alpha$ /Akt/ERK pathway	(91)
LncRNA-UCA1/miR-873-5p	Human MSCs	Up-regulated/down-regulated	Antiapoptotic and therapeutic effects	miR-873-5p/XIAP axis	(92)
miRNA-21	Human MSCs	Up-regulated	Promote maxillofacial bone regeneration	PTEN/PI3K/Akt/HIF-1 $\alpha$	(93)
CircCDK8	PDLSCs	Up-regulated	Repress the osteogenic differentiation	ER stress/autophagy	(94)
LncRNA DN3M3OS/miR-127-5p	Rat MSCs	Up-regulated/down-regulated	Inhibit chondrogenic differentiation	DNM3OS/miR-127-5p/GREM2 axis	(95)

studies were shown that the differentiation ability of BMSCs is reduced under hypoxia compared with normoxia, supporting the idea that hypoxic condition promotes the undifferentiated state of BMSCs (26, 101). In conclusion, oxygen tension is an important factor affecting cell fate and regulating cell state. The expressions of OCT-4 and REX-1 are increased in human MSCs under chronic hypoxia (2% O<sub>2</sub>) and enhances colony forming unit-fibroblast (CFU-F) capacity while preserving their ability to differentiate into osteoblasts or adipogenic (76).

Hypoxia (3% O<sub>2</sub>) was also used to isolate marrow-isolated adult multilineage inducible (MIAMI) cell populations from pluripotent human bone marrow (102). Hypoxia affects the differentiation characteristics of BMSCs, which may be related to the physiological oxygen demand of differentiated cells. It was found that hypoxia enhanced *in vitro* and *in vivo* osteogenic potential of rat MSCs (35), while inhibited *in vitro* osteogenic potential of MIAMI cells (26), and down-regulate osteogenic genes *in vitro* (103). The angiogenic ability of mouse MSCs was enhanced under 2% O<sub>2</sub> (22), and the expression levels of chondrogenic markers SOX-9, aggregative proteoglycan and Col IIa were increased under 1% O<sub>2</sub> (37). Hypoxia (1% O<sub>2</sub>) for 24 h induced the formation of lipid droplets in human MSCs, but did not up-regulate the expression of adipocyte genes (29).

Numerous studies have shown that miR-210 promotes cell differentiation. Hypoxia-induced miR-210 may promote osteoblast differentiation by inhibiting ACVR1B-Smad 2/3 and activating Smad 1/5/8 (104). Hypoxic-associated miR-21 promotes angiogenesis of human umbilical cord blood derived MSCs (UCBMSCs). miR-21 has been demonstrated to promote osteogenesis and enhance osteogenic regeneration of critical size defects through the PTEN/PI3K/Akt/HIF-1 $\alpha$  pathway (93). LncRNA UCA1 of MSCs derived exon plays a protective role through miR-873-5P/XIAP axis in hypoxia (92). CircCDK8 inhibits PDLSCs osteogenic differentiation by

triggering autophagy activation in hypoxic microenvironment (94). The relevant ncRNAs involved in regulating the fate and regeneration of MSCs under hypoxia were listed in **Table 2**.

## DISCUSSIONS

One of the striking features of mammalian development and of human physiological and pathological conditions is hypoxia-mediated regulation of stem cell fate, or a decline in oxygen utilization. Therefore, oxygen sensing is necessary because it allows cells to instantly adapt to inadequate amounts of potassium dihydrogen phosphate. HIF is the main regulatory mechanism. In addition, there is increasing evidence that the hypoxic microenvironment is the niche of most types of stem cells, which may be beneficial for stem cell maintenance and the continued replacement of dead or damaged cells in body tissues (105).

The optimal oxygen concentration for stimulating differentiation and survival of stem cells is uncertain and may be influenced by other culture conditions (69). Physiologically normoxic oxygen is considered hypoxic because there is a conserved molecular reaction in the oxygen tension range of 2-9%. Molecular channels that may be involved include HIF, oxygen ion channel, rapamycin target (mTOR) and endoplasmic reticulum stress response (106–108). As important regulators of stem cell dryness, Oct4 and Notch signals have also been confirmed to be activated by hypoxia in a variety of stem cells (109). Other studies have shown that different degrees of hypoxia have different effects on the proliferation and differentiation of stem cells, suggesting that the proliferation or stasis of stem cells can be regulated by the oxygen concentration of local ecological niche. For example, proliferation of ESCs is inhibited and differentiation increases at oxygen concentrations

as low as 1%, while pluripotency of ESCs is maintained at 3%-5% oxygen tension without significant change in proliferation (110).

Hypoxia and epigenetic interactions have potential applications in regenerative medicine. It is reported that reprogramming cells exist in many different types of tissues and organs after hypoxia (111, 112). Studies have shown that hypoxic environment may contribute to or regulate the process of tissue regeneration. Phenomena such as conditional ischemia caused by hypoxia stimulation can provide attractive prospects for future research (113–115).

As a key factor in determining the characteristics of BMSCs, the microenvironmental niche could maintain and regulate their function. Cell growth and differentiation require a certain amount of oxygen. However, under some physiological and pathological conditions, hypoxia is common. Under extreme hypoxia, the oxygen concentration can be reduced to <1% (116). Oxygen can produce ATP, a product necessary for cell survival. Through oxidative phosphorylation of mitochondria, cells maintain an adequate supply of ATP. However, under hypoxia, cells promote HIF activation or reduce gene transcription by sensing the change of oxygen tension. Under the premise of insufficient oxygen, the adaptive process is activated to improve cell survival (117).

On the one hand, hypoxia preconditioning enhances the osteogenic differentiation potential of BMSCs (31, 118), which could promote bone regeneration (25) and adipogenic differentiation (77). While short-term (48 h) hypoxic exposure (1% O<sub>2</sub>) suggest decreased osteogenic differentiation after transplantation of BMSCs to a hypoxic microenvironment (103). It was showed that hypoxia reduces osteogenic and adipogenic differentiation of MSCs by inhibiting RUNX2 (41) and HIF-1 $\alpha$  stabilization (24, 118, 119). It is reported that early hypoxia can accelerate the osteogenesis of BMSCs, however, prolonged hypoxia results in the opposite trend (27). Nevertheless, it was shown that hypoxia culture may not exert effect on the osteogenesis of BMSCs (120, 121).

In view of the practical significance of hypoxia in the development and differentiation stage of stem cells, the outcome of stem cell fate and differentiation of research has been underway for more than 20 years. However, due to the differences of cell separation methods, research parameters, growth factors, oxygen concentration and evaluation experimental techniques, the differences between the research results of the role of oxygen in stem cells are highlighted (122). There are still a lot of unknown and exploratory areas in the later research. With the development of transcriptomics and bioinformatics, the behavior of stem cells focuses on epigenetic regulation and modification, and hypoxia has an effect on the epigenetic modification levels of cells. The coexistence of transcription factors suggests the correlation between hypoxia and cell reprogramming related epigenetics. More and more studies have focused on the roles of lncRNAs, circRNAs and miRNAs and their interactions in the regulation

of stem cell survival and regeneration under hypoxia. The network could help us better interpret stem cell behavior and provide direction for *in vitro* modification of stem cells for transplantation.

MSCs can be collected from multiple parts of the oral cavity, such as the dental papilla, dental pulp, periodontal ligament, etc. These stem cells can be used as good tools for bone regeneration and tooth tissue regeneration. Odontogenic stem cells are hypoxic *in vivo* under both physiological and pathological conditions. The average oxygen partial pressure in the pulp of rat incisors is 23.2 mmHg, which is approximately equivalent to 3-12% oxygen concentration (116, 123, 124). In some relatively hypoxic tissues or pathological conditions, oxygen concentration is lower, often less than 1% (116). The pulp is wrapped by the hard dental tissue, and blood oxygen can only reach the pulp cells through the narrow apical orifice, lacking effective collateral circulation. When inflammation or trauma occurs, the vascular nerve bundles of the pulp are easily damaged, resulting in the state of ischemia and hypoxia of the pulp (125). Under the condition of periodontal inflammation, the deeper the periodontal pocket, the lower the oxygen content. The research shows that the average oxygen partial pressure in the deep periodontal pocket is only 1.8% O<sub>2</sub> (126). Hypoxia is a common phenomenon in both pathological and physiological states. Currently, hypoxia-related ncRNAs (lncRNA STL, miR-210, lncRNA H19, circRNA CDK8) have been thought to be related to the fate of odontogenic stem cells through different mechanisms. More differentiated ncRNAs induced by hypoxia are worthy of further study and exploration as potential targets.

In addition, oxygen, as a metabolic substrate, can correspondingly reshape cell metabolism. HIF pathway, as a transcription factor, participates in cell metabolism, making it the main contributor to cell response during hypoxia, energy crisis and metabolic disorder. In turn, HIF pathway activity is regulated by various factors, including the supply and utilization of O<sub>2</sub> and key metabolic substrates (127–129). Hypoxia induced HIF activity initiates a powerful transcriptional program of more than 200 genes. Broadly speaking, the purpose of these adaptations is to increase oxygen delivery or reduce oxygen consumption to meet metabolic needs and normalize local PO<sub>2</sub>. For example, HIF induction triggers erythropoiesis [erythropoietin (EPO)] and iron homeostasis [e.g., transferrin (TF)] to increase circulating red blood cell (RBC) levels (130, 131). The behavior and differentiation fate of MSCs are closely related to metabolism. As an important substrate of cell metabolism, hypoxia is involved in regulating the behavior and fate of MSCs by affecting ncRNAs, which is worthy of further exploration.

## CONCLUSIONS

As the physiological environment of stem cells, hypoxia also exists in the tumor microenvironment, inflammatory microenvironment and temporary stimulation of some extreme

organism conditions, which has the function of double-edged sword. The effect of hypoxia on MSCs may be closely related to the duration of treatment, oxygen concentration, and cell state. There are also differences in regulatory mechanisms among different cells. NcRNAs have played a significant role in the modulation of hypoxia on MSCs. Studies of ncRNAs and their interaction can promote or reverse the effects of hypoxia microenvironment on MSCs, and provide good conditions for better modified stem cells for *in vivo* transplantation.

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## AUTHOR CONTRIBUTIONS

YY: drafted the manuscript and revised. XZ: collected and integrated data. YX and JY: conceptualization and funding acquisition. All authors read and approved the final manuscript.

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