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The Role of Twist1 in Stem Cell Differentiation through Mechanical Cues: A Review and Hypothesis

Yinqiu Yan[1], Zeyun Tian[1], Qiuyue Guan[2], Ding Bai[1], Jing Zhang[3*] and Xianglong Han[1*]

[1]Department of Orthodontics, State Key Laboratory of Oral Disease, West China School of Stomatology, Sichuan University, Chengdu, P.R. China.
[2]Department of Geriatrics, People's Hospital of Sichuan Province, Chengdu, P.R. China.
[3]Department of Orthodontics, Sixth People's Hospital, Anyang, P.R. China.

Authors' contributions

This work was carried out in collaboration among all authors. Author XH designed the study. Author YY wrote majorly to the draft of the manuscript. Author ZT helped with figures in this paper. Authors QG, DB, XH and JZ assisted in literature reviewing and amendment. All authors read and approved the final manuscript.

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ABSTRACT

Due to the pivotal role of stem cell differentiation in regeneration and disease cure, the study of it has always been a research highlight during the recent years. Stress microenvironment has a great impact on cell growth, proliferation, differentiation and apoptosis. Twist1, as a core epithelial-mesenchymal transition (EMT) regulatory factor, plays an important role in these processes. Moreover, Twist1 gene can express in alveolar bone – periodontal ligament interface and the expression can be regulated by changes in the occlusal force. In this article, we will present a review of Twist1 gene, especially in the aspect of the biological functions in stem cell differentiation under mechanical signals and explore whether Twist1 involved in tissue remodeling in alveolar bone - periodontal membrane interface under stress.

**Corresponding author: E-mail: xhan@scu.edu.cn;*

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1. INTRODUCTION

Twist1 is a basic helix-loop-helix transcription factor and originally isolates as a mutant in Drosophila [1]. The name "Twist" given to this gene is on the basis that Drosophila embryos lacking the Twist1 gene could not gastrulate normally, formed no mesoderm and finally died at the end of embryogenesis, leaving a 'twisted' appearance [1,2]. Accumulating functional and genetic studies both *in vitro* and *in vivo* have indicated that Twist1 is an important physiological modulator of mesenchymal cell fate during epithelial–mesenchymal transition (EMT), tumor initiation, and progression. Notably, some mechanisms of action of Twist1 in mesenchymal cell differentiation have been recently identified, especially in mesenchymal cell lineage allocation and skeletogenesis [3]. Besides, the diseases such as the Saethre-Chotzen syndrome induced by human Twist1 insufficiency suggested the vital role of Twist1 in the pathogenesis of human craniosynostosis [4]. Moreover, Twist1 is strongly expressed in the periodontal ligament and Twist1 mRNA expressions are regulated by altered occlusal force [5]. As the structure and function of the alveolar bone - periodontal membrane interface are closely related to occlusal function, we considered Twist1 might play a potential role in the interface under stress.

This review summarizes the recent knowledge on the classification, structure, tissue/cell expression and biological functions of Twist1, with specific emphasis on biological roles of it in control of cell fate under mechanical cues. As Twist1 is a pivotal transcriptional factor in the alveolar bone - periodontal membrane interface, we also offer a hypothesis that this factor may act in the remodeling of this interface under stress microenvironment. This points to the significant contribution of Twist1 in osteoblastogenesis and the differentiation of periodontal ligament stem cells, which may provide therapeutic perspectives and potential applications in the periodontal diseases, peri-implant hard tissue defects, orthodontic tooth movement as well as other associated biomechanical studies in periodontal tissue remodeling.

2. TWIST1 IS A CORE REGULATORY TRANSCRIPTION FACTOR

2.1 Distribution, Structure and Classification of Twist1

As a vital gene for organogenesis, Twist1 is strongly expressed in the mesoderm-derived embryonic mesenchyme. In postnatal tissues of species including Drosophila, mouse and human, Twist1 is predominantly distributed in adult stem cells located in mesoderm-derived tissues such as the adipose tissue, bone marrow and skeletal muscle [1,6]. Twist has been classified as a NC(neural crest) specifier in invertebrate creatures and research practiced on Xenopus suggested that Twist is different from other NC specifiers by the limitation of its expression to cranial regions. This localization demonstrates that Twist might play a role in promoting cranial NC precursors to give rise to mesectodermal derivatives, such as cartilage and bone [7].

Three motifs have been demonstrated due to structural consideration of the Twist protein, which include a basic domain, a helix–loop–helix (HLH) domain and a C-terminal domain, the WR domain or Twist box. The basic domain can mediate interaction with DNA, especially with the core E-box sequence 'CANNTG'. The HLH motif, firstly identified in the murine DNA-binding proteins E12 and E47, is necessary and sufficient for protein dimerization or homodimerization and shows a higher degree of identity among vertebrates (Fig. 1) the WR domain is located between 20 and 55 amino acids C-terminal in the bHLH region and highly conserved within vertebrates and jellyfish. Recent research has shown that it may mediate physical interactions between Twist and other core epithelial–mesenchymal transcriptional factors, for instance Snail1 and Snail2, which are essential for appropriate functions [7,8]. Further studies have shown the WR motif can interact with another non-bHLH transcription factor, Runx2, to impede osteoblast-specific gene expression and similarly inhibit Sox9 activity during chondrogenesis, as well as an activation domain for Twist-E12 dimers. Phosphorylation of multiple GSK3-beta sites in the Twist C-terminus, which have already been identified, is necessary for Twist function and for its restraining interactions with Snail proteins [7,9,10].

The traditional classifications of bHLH proteins are based on their tissue distribution, partner choice, DNA-binding performances and structural features, which categorizes the bHLH family into three subfamilies: Class A, class B and class C. The proteins in class A, including E12, E47, HEB, E2-2 and Daughterless, are broadly expressed in mammalian cells. Class B contains bHLH proteins that have relative tissue specificity and constitute dimers with class A molecules. Class C molecules, comprising the Myc proteins, do not organize heterodimers with either class A or class B proteins. The Twist family belongs to class B, as it has specificity in tissue expression and constitutes heterodimers with E12 and E47 [1,8]. Twist1 and Twist2 are highly homologous bHLH transcription factors of twist family. They appear to exhibit highly overlapping expression patterns during development. While both proteins have been revealed to impede osteogenesis, only Twist1 haploinsufficiency is related to the premature synostosis of cranial sutures in mice and humans [11].

2.2 Diverse Functions of Twist1 in Development and Pathological Processes

Different classes of bHLH proteins, as well as different dimer forms, act as either positive or negative transcriptional regulators. Twist1 can form homodimers and heterodimers with the E protein family and individual homodimer and heterodimer bHLH combinations can result in differences in DNA binding affinity, target preference site, and biological activities, demonstrating that partner choice is an additional key regulation point for bHLH proteins [8,12]. For instance, depending on dimer partner, Twist1 acts either as an activator or a repressor of somatic muscle development. Twist1 homodimers specify mesoderm and the subsequent allocation of mesodermal cells to the somatic muscle fate, while heterodimers of Twist1 and Daughterless repress genes required for somatic myogenesis [12,13].

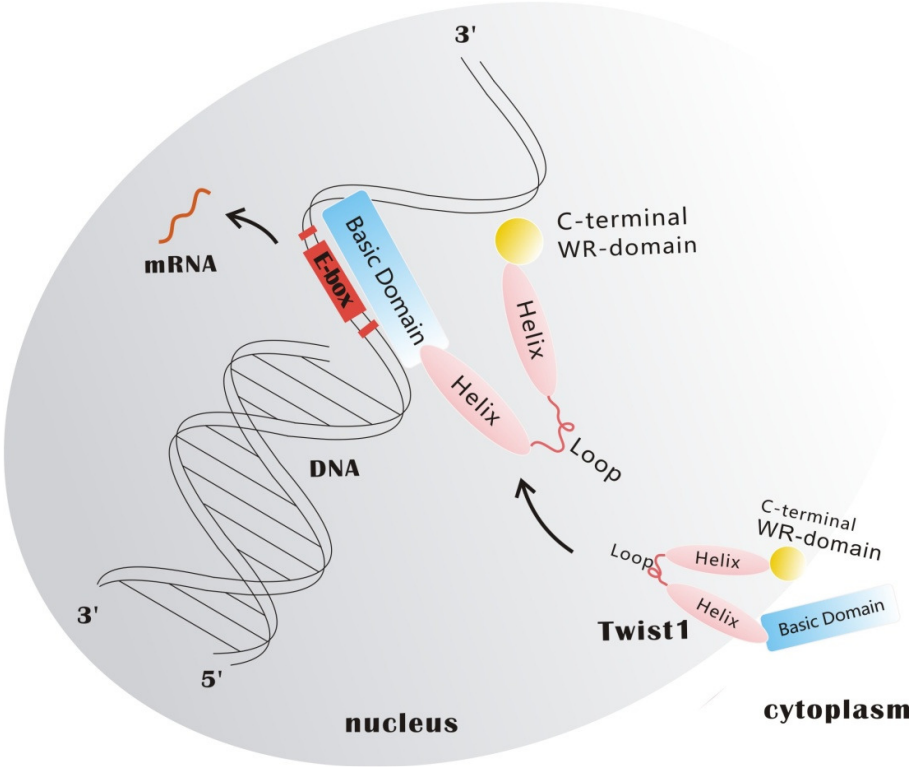

Fig. 1. The structure of twist protein includes a basic domain, a helix–loop–helix (HLH) domain and a C-terminal domain. In the cell nucleus, the Twist's basic domain interacts with the E-box of DNA to function as a transcription regulator. The HLH motif is required for protein dimerization or homodimerization. The C-terminal WR domain physically interacts with transcription factors for transcriptional regulation. mRNA: Messenger RNA

Twist1 is essential for mesoderm specification and differentiation during development [1]. During Drosophila development, Twist1 controls a sequence of important decisions within the same lineage as a multifunctional protein [12]. Different functions have been clarified when Twist1 acts in different stages. These functions of Twist1 are reflected in its dynamic pattern of expression, which is characterized by initial uniform expression during mesoderm induction to activate a number of mesoderm-specific regulators, such as the FGF receptor heartless (htl), DMef2, and snail, then followed by modulated expression at high and low levels in diverse mesodermal segment, for instance, high levels of Twist1 expression in tissues like visceral mesoderm and leads to formation of ectopic body muscles while expression of Twist1 at low levels interferes with somatic myogenesis but permits the development of other tissues [8,12]. Diversities of the molecular mechanisms by which Twist1 executes the function of mesoderm induction and proper differentiation of a subset of mesodermal tissues, especially muscles have also been found in a variety of organisms. For example, Whereas Drosophila Twist (DTwist) is necessary for the induction of mesoderm and gastrulation, the vertebrate Twist gene is not detected prior to gastrulation but after the mesoderm is specified. In addition, although Twist promotes myogenesis in Drosophila, Twist appears to play an opposite role in vertebrate myogenesis [8].

In amniotes, although Twist1 may not have a role as a NC specifier, it can function as a core epithelial–mesenchymal transition (EMT) regulatory factor in both developmental and pathological contexts like Snail1/2 [7]. Heterozygous loss-of-function mutations in the human Twist1 gene can cause several diseases including the Saethre-Chotzen syndrome (dominantly inherited condition that includes a variety of physical abnormalities, including craniosynostosis and subtle facial and digital anomalies, such as ptosis, tear duct anomalies, syndactyly and polydactyly of the hands and feet) [2,4]. In addition, the Twist1-null mouse embryos die with unclosed cranial neural tubes and defective head mesenchyme, somites, and limb buds. Twist1 is implicated in the EMT/progression of multiple epithelial cancers, including breast cancer, hepatocellular carcinoma, prostate cancer, gastric cancer and its expression is mostly related to invasive and metastatic cancer phenotypes. In cancer cells, numerous factors including SRC-1, STAT3, MSX2, HIF-1α, NF-κB and integrin-linked kinase can upregulate Twist1. Twist1 can also increase cell proliferation and the ability to evade apoptosis in aggressive tumor cells. Expression of Twist1 in primary tumor cells has been shown to override oncogene-induced cellular senescence and apoptosis and has been linked to the maintenance of a 'cancer stem cell' state [1,7].

3. STRESS MICROENVIRONMENT PLAYS AN IMPORTANT ROLE IN STEM CELL DIFFERENTIATION

Mechanical cues from the extracellular microenvironment play a key role in regulating the structure, function and fate of living cells [14]. Recent work has verified that both internal and external physical forces can act through the cytoskeleton to affect cellular behavior [15]. Further studies demonstrated mechanical stimuli which are associated with direct mechanical strain or osmotic stress are converted to intracellular signals that control cellular physiology in both health and disease, and this process is called mechanotransduction. In the process, extracellular mechanical forces can activate plenty of well-studied cytoplasmic signaling cascades and then such signals are transduced to the nucleus and induce transcription factor–mediated changes in gene expression. Moreover, remodeling of the nucleus induces alterations in nuclear stiffness, which may be associated with cell differentiation. In other words, nuclear architecture-mediated mechano-regulation has the potential influence of transcription and cell fate [16].

3.1 Multipotent Differentiation of Periodontal Stem Cells under Stress Microenvironment

Stem cells are related to a wide variety of cells from different sources and they can be generally divided into two categories – embryonic and adult. Embryonic stem cells are totipotent cells, capable of differentiating into virtually any cell type, as well as being indefinitely an undifferentiated state while adult stem cells can be further classified depending on their origin and differentiation potential. Two common examples are hematopoietic and mesenchymal stem cells(MSCs) [17]. And recently, multiple stem cells including embryonic stem cells (ESCs), bone marrow-derived mesenchymal stem cells (BM-MSCs) and dental pulp stem cells

(DPSCs) have received widespread attention in the field of bone tissue engineering owing to their biological capability to differentiate into osteogenic lineages. During the process in vivo embryogenesis, totipotent ESCs initiate early differentiation into three primary germ layers through gastrulation, which are ectoderm, mesoderm, and endoderm. It has been well verified that osteogenic lineage cells are derived from the somatic mesoderm or the ectomesenchymal cells of the neural crest. These cells are considered to differentiate from the mesodermal progenitor cells or mesenchymal progenitor cells [18].

Five different human dental stem cells including periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs), dental follicle progenitor cells (DFPCs), stem cells from exfoliated deciduous teeth (SHED), and stem cells from apical papilla (SCAP) have been isolated and characterized. These progenitor cells have mesenchymal stem cell-like (MSC) qualities as they contain the capacity for self-renewal and multi-lineage differentiation potential [19]. Research has demonstrated that overexpressing or silencing Twist1 levels with lentiviral vectors could alter the differentiation capacity of DPSCs [20]. Under defined culture conditions, PDLSCs exhibit osteogenic, adipogenic, and chondrogenic characteristics and can differentiate into many pathways, including dentinogenic lineage, mesodermal lineage, ectodermal lineage and even vascular lineage [19,21,22]. Several studies suggested that periodontal ligament cells have the ability to adapt to their functional environment and mechanical vibration can enhance the osteogenic differentiation of periodontal ligament stem cells depending on frequency [21,23]. As the dominant cells of the periodontal ligament, fibroblasts play an essential role in response to mechanical force loading of the tooth by remodeling and repairing matrix components [21].

3.2 Bone Cell Differentiation and Bone Remodeling under Mechanical Cues

Bone remodeling is a physiological process of bone resorption and bone formation, which is carried out by a functional and anatomic structure known as ‘basic multicellular unit’ (BMU) [24]. Four types of bone cells act harmoniously in the bone remodeling process, including bone lining cells, osteocytes, osteoclasts and osteoblasts. Bone lining cells form a monolayer covering the bone surface in a still state. Osteocytes, the most abundant and representative bone cells, embedded within the bone during skeletal maturation or previous cycles of bone remodeling, play a role as the primary mechanosensing cell and may initiate the process of bone remodeling. Osteoclasts and osteoblasts are the bone resorbing cells and bone-forming cells, respectively. Whereas multi-nucleated osteoclasts are of hematopoietic origin and differentiate from mononuclear cells of the monocyte/macrophage lineage, osteoblasts are derived from mesenchymal stem cells (MSC). The bone line cells and osteocytes belong to the osteoblast lineage as well. In the BMU, osteoclasts remove the bone and prepare the site for the following bone-forming osteoblasts. The human adult skeleton has about 1-2 million active BMUs at any given time, which are temporally and spatially separated from each other and functional asynchronously [25,26].

The mechanosensitivity of gene expression is common in mammalian cells submitted to constant mechanical stresses, which include osteoblasts and the endothelial cells that line blood vessels. In osteoblasts, mechanical stress on the plasma membrane restrains the endocytosis of BMP2 and induces the nuclear translocation of a Smad-family transcription factor, which triggers and maintains osteoblast differentiation [27].

4. POTENTIAL ROLE OF TWIST1 IN THE ALVEOLAR BONE-PERIODONTAL LIGAMENT INTERFACE REMODELING UNDER STRESS MICRO-ENVIRONMENT

Earlier gene ablation experiments have shown that Twist1 is necessary for closure of the neural tube during mouse development [28]. Besides, Twist1+/- mice present a craniosynostosis phenotype such as an increased bone formation in cranial sutures. The same phenotype is also observed in Saethre-Chotzen patients in human [2,29,30]. These observations suggested the capability of Twist1 to inhibit osteoblast differentiation. Furthermore, Twist1 is believed to maintain mesenchymal cells in an undifferentiated state by negatively regulating Runx2, which is a vital osteoblast-differentiation transcription factor essential for bone formation, and may perform its antiosteogenic functions, by the interaction with the Runx2 DNA-binding domain, while Twist2 and Runx2 are believed not

coexpressed in the developing skull. Moreover, it's believed that the lack of sufficient Runx2 inhibition by Twist1 in Twist1+/– mice leads to the premature differentiation of odontoblasts, resulting in the formation of extensive pulp stones in the tooth [7,10,31]. In addition, FGF signaling plays essential role throughout osteogenesis and further support was provided that Twist1 and Twist2 regulate FGF signaling in bone formation. In a compound Twist1- and Twist2-haploinsufficient animal model, reduced bone formation and impaired proliferation and differentiation of osteoprogenitors are exhibited rather than premature ossification and craniosynostosis. However, it remains to be determined why only Twist1/E12 heterodimers, instead of Twist1 homodimers, stimulate Fgfr2 promoter activity [11]. Overall, various molecular mechanisms may contribute to the inhibitory role of Twist1 in osteoblast differentiation. Twist1 may modulate FGF signaling, especially Fgfr2 expression in cranial suture development or it may directly bind to and suppress the transactivation function of Runx2. In addition, Twist1 might indirectly regulate the Runx2 expression through modulating Fgfr2 expression. Finally, it is possible that Twist1 restrains osteoblast apoptosis via the inhibition of TNF-α expression [11,32,33] (Fig. 2.C).

Recent studies have confirmed that the biological function of Twist1 is closely related to the composition ratio of homodimers and heterodimers. We have known that Class B bHLH factors can constitute functional homodimers and heterodimers with Class A bHLH factors including E12/E47. Id proteins have a great affinity for E12 and compete against class B bHLH proteins for E12 binding. Twist1 and E12 proteins can form heterodimers to inhibit FGFR2 and maintain osteoblasts in an undifferentiated state by directly bonding fibroblast growth factor receptor -2 (fibroblast growth factor receptor 2, FGFR2) promoter in osteoblasts. Id protein may compete with Twist1 for binding E12 protein, and as a result, the formation amount of Twist1 homodimers increases. Then, Twist1 homodimers activated FGFR2 to indirectly regulate Runx2 transcription, thereby promoting osteoblast differentiation and maturation. Thus, by modulating the proliferation, differentiation, and apoptosis of osteoblasts, Twist1 may be involved in the craniofacial bone remodeling process [33,34].

The highest expression of Twist1 mRNA was detected in mouse PDL cells when compared to other oral tissues suggesting some physiological role for Twist1 in the PDL. A real-time PCR

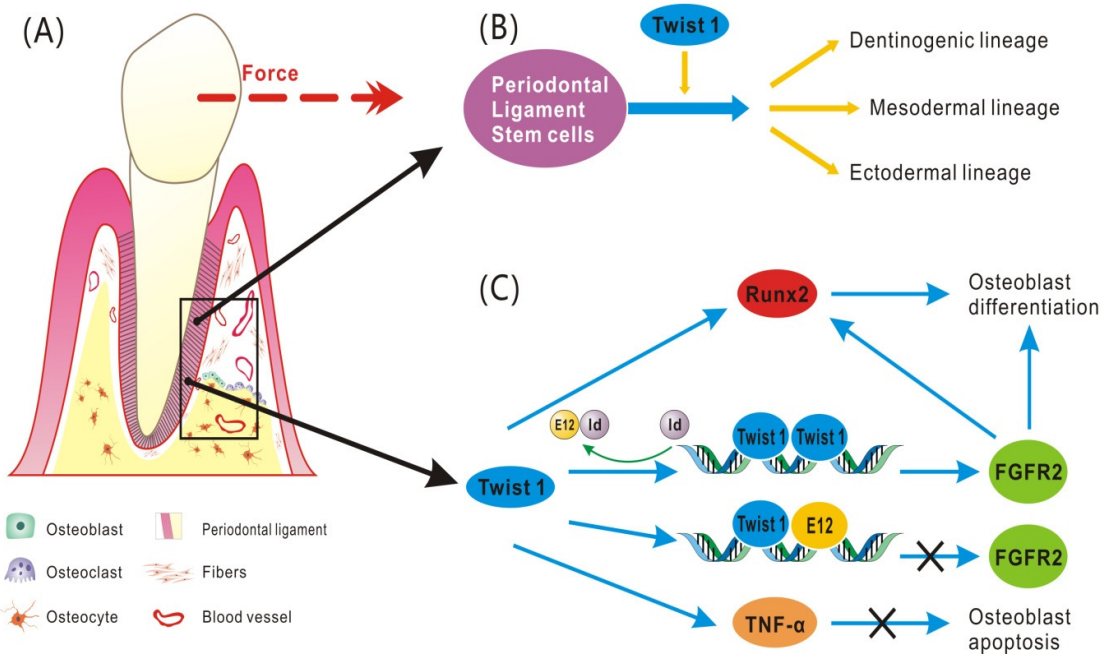

Fig. 2. (A) Alveolar bone-periodontal ligament interface under stress microenvironment. (B) Differentiation potential of periodontal ligament stem cells under force and the role of Twist1. (C) Modes of transcriptional regulation by Twist 1 in osteogenic differentiation

analysis showed the transient decrease of periostin and Twist1 mRNA caused by occlusal hypofunction in mouse periodontal ligament. These results showed occlusal force might impact on periostin and Twist1 gene expression in the PDL and the changes in their expression level may be considered a form of adaptation to environmental changes [5]. In another experiment, by applying unilateral compression to whole drosophila embryos, Twist1 is demonstrated to ectopically express in response to a uniaxial compression of the early embryo. Besides, Twist1 induction by form change depends on the nuclear translocation of armadillo (a transcriptional coactivator and component of the cadherin complex that is homologous to human beta–catenin). Furthermore, the cells in the mutant flies exhibited abnormally low Twist1 expression, and mechanical compression can rescue that expression which shows that mechanical cues can be transduced by Twist1 gene response [27,35]. S. Premaraj et al. [36,37] found that β-catenin signaling responds to mechanical loading in periodontal ligament (PDL) cells. The stabilization and activation ofβ-catenin pathway in that occasion are occurred by FAK-mediated activation of PI3K/ Akt pathway and mechanical loading-induced NO production. L. H. Goodnough et al. [38] demonstrated that activation of β-catenin signaling is required and sufficient for Twist1 expression. A genetic interaction *in vivo* betweenβ-catenin and Twist1 was also suggested and this kind of interaction is required to ensure appropriate osteoprogenitor fate in intramembranous bones of the skull.

On the basis of the results and analyses mentioned above, we suppose that Twist1 may play a role in the alveolar bone-periodontal ligament interface remodeling by impacting on stem cell differentiation under mechanical signals. (Fig. 2) However, the exact mechanism of the possible pathways is not well understood.

5. CONCLUSION

Growing evidence has demonstrated the important roles of Twist 1 in cell differentiation. Several specific pathways and the genes or factors involved are unraveled as well. However, how will Twist1 work in the alveolar bone-periodontal ligament interface and what is the difference of the mechanism when compared to other tissues of the organism are still puzzles to be explored. Besides, since the periodontal ligament stem cells are considerable resources for regeneration, the possibility of Twist1 as an applicable target gene will be a promising issue and might provide new and effective strategies for regenerative dentistry. Furthermore, the oral cavity is a congenital stress microenvironment, so how the mechanical cues initiate and modulate the cellular processes when Twist1 involved will be of great value in some related biomechanical research, such as tooth movement in orthodontics.

Nevertheless, critical barriers for the application of these findings into human clinical trials need to be considered. Therefore, further studies are necessary to clarify these factors, which are responsible for the successful outcome of stem cell-based tissue remodeling.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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