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Stem Cells in Teeth and Craniofacial Bones

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Abstract

Stem cells are remarkable, and stem cell–based tissue engineering is an emerging field of biomedical science aiming to restore damaged tissue or organs. In dentistry and reconstructive facial surgery, it is of great interest to restore lost teeth or craniofacial bone defects using stem cell–mediated therapy. In the craniofacial region, various stem cell populations have been identified with regeneration potential. In this review, we provide an overview of the current knowledge concerning the various types of tooth- and craniofacial bone–related stem cells and discuss their in vivo identities and regulating mechanisms.

Keywords: mesenchymal stromal cells, neural crest, hedgehogs, Gli1 protein, skull, stem cell niche

Introduction

Stem cells are characterized by their continuous self-renewal and multipotential differentiation capabilities. The existence of stem cells was first demonstrated in the hematopoietic system with studies showing that cells from the bone marrow can give rise to multilineage blood descendants while retaining their self-renewal ability (McCulloch and Till 1960). The same bone marrow tissue was subsequently shown to host a stromal type of multipotential cell—namely, mesenchymal stem cells (MSCs; Friedenstein et al. 1968). Since these pioneering studies, stem cells have become a popular topic for researchers and the general public, due to their remarkable ability to regenerate damaged tissue and treat certain diseases. Various tissue-specific stem cells have been identified in different organs.

Craniofacial tissue damage, including bone fractures and tooth loss, presents a major challenge for dentists and craniofacial surgeons. Craniofacial damage has a particularly negative effect for patients, because the face often represents our identity. For much of the history of these fields, defective bones or missing teeth could be replaced only with artificial prostheses, which can never completely restore the physiologic functions of natural organs. The concept of stem cell therapy provides a promising approach to designing new therapies for functional restoration. Although hematopoietic and MSCs were first identified >40 y ago, the study of stem cells in the dental and craniofacial field has lagged behind the study of stem cells in other tissues. The existence of dental stem cells was first demonstrated in the early 21st century (Gronthos et al. 2000). The major concepts and mechanisms in early dental and craniofacial stem cell studies were mostly borrowed from previous bone marrow MSC research. In the current review, we focus on the latest progress in studying bone marrow MSCs as well as dental and craniofacial MSCs. In addition, we briefly introduce research on dental epithelial stem cells due to their close association with dental MSCs.

Bone Marrow MSCs

MSCs were first identified in studies by Alexander Friedenstein and colleagues in the 1960s at the University of Moscow, which laid the foundation for subsequent MSC studies (Friedenstein et al. 1968). Friedenstein's team discovered that bone marrow contains fibroblast-like stromal cells that are capable of forming clones and differentiating into osteocytes. Later studies indicated that similar populations are also capable of differentiating into adipocytes and chondrocytes (Friedenstein et al. 1982; Friedenstein et al. 1987; Mardon et al. 1987; Owen and Friedenstein 1988). The term *mesenchymal stem cells* was first adopted in the 1990s to define bone marrow stromal cells that can adhere to the culture plate and are capable of multipotential differentiation (Caplan 1991). Besides adipocytes, chondrocytes, and osteocytes, bone marrow MSCs are also able to differentiate into skeletal myocytes and tenocytes, among other cell types (Awad et al. 1999; Gang et al. 2004).

The study of MSCs has been profoundly influenced by earlier studies of hematopoietic stem cells. Because a number of surface markers were identified to aid in the identification and isolation of hematopoietic stem cells, similar approaches were adopted to identify MSCs. Many surface markers—including CD271, CD146, CD90, and CD105—have been used to identify MSCs (Cattoretti et al. 1993; Aslan et al. 2006; Sacchetti et al. 2007; Mabuchi et al. 2013; Kfoury and Scadden 2015). In 2006, the International Society for Cellular Society Therapy

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proposed a standard for defining MSCs as multipotential stromal cells that meet the following criteria:

1) they can attach to and grow on an uncoated culture dish;
2) they strongly express markers, including CD90, CD73, CD105, and CD44, but are negative for hematopoietic markers, such as CD34, CD45, CD11B, and CD19; and
3) they possess trilineage differentiation ability (osteogenic, adipogenic, and chondrogenic) in vitro, subject to appropriate conditions (Horwitz et al. 2005).

This definition is based on the in vitro properties of cultured cells, and it remains largely unknown whether the same criteria can be used to identify MSCs in vivo.

The use of transgenic mouse models has had a significant impact on the study of MSCs. Lineage tracing techniques enable the labeling of stem cells in a temporal and tissue-specific fashion. Animals with a fluorescent or LacZ reporter under the control of regulatory genetic elements critical for skeletal or mesenchymal development have been used for these studies (Table).

Nestin+ cells were first proposed to be the in vivo counterparts of bone marrow MSCs (Mendez-Ferrer et al. 2010). In the bone marrow, *Nestin*+ cells are a rare nonhematopoietic stromal population with a perivascular distribution. Isolated *Nestin*+ cells are capable of forming clones and have a robust self-renewal potential even after serial transplantation. Lineage tracing experiments based on the *Nestin-CreERT* model indicate the contribution of *Nestin*+ cells to osteochondral tissue during injury repair.

Mx1 was also proposed to label bone marrow MSCs (Park et al. 2012). *Mx1*+ cells are mostly perivascular and partially overlap with *Nestin*+ cells. They express typical MSC markers, such as CD105 and Sca1. *Mx1*+ cells exhibit enriched clonogenic ability and trilineage differentiation ability in vitro. Lineage tracing experiments indicate that *Mx1*+ cells contribute to bone formation during the repair of fracture injury. However, in addition to perivascular MSCs, *Mx1* robustly labels hematopoietic cells, which makes it an inappropriate model for studying bone–hematopoietic stem cell interactions.

Leptin Receptor (*LepR*) was recently proposed to be an enriching marker for bone marrow MSCs (Zhou et al. 2014). Approximately 0.3% of bone marrow cells are *LepR*+, but they account for 94% of clonogenic ability in vitro. Lineage tracing experiments indicate that *LepR*+ cells can give rise to bone, cartilage, and adipocytes. They also make a significant contribution to new tissue formation during injury repair. Interestingly, *LepR*+ cells do not express *Nestin*. They are perivascular, arise postnatally, and give rise to most bone and adipocytes formed in adult bone marrow during postnatal development.

In addition, the BMP antagonist *Gremlin1* was proposed to define MSCs in vivo (Worthley et al. 2015). *Gremlin1*+ cells are also perivascular stromal cells and do not express *Nestin*. They have a restricted ability to differentiate into osteoblasts, chondrocytes, and bone marrow stromal cells but do not differentiate into adipocytes. In vitro, *Gremlin1*+ cells possess robust clonogenic and trilineage differentiation ability.

Most recently, *Gli1* was proposed to be a universal marker for MSCs in various organs, including the kidney, lung, liver, heart, tooth, and bone (Zhao et al. 2014; Kramann et al. 2015; Zhao et al. 2015). In vitro, *Gli1*+ cells highly express typical MSC markers, exhibit trilineage differentiation capacity, and possess colony-forming activity. Genetic lineage tracing analysis demonstrated that tissue-resident *Gli1*+ cells rapidly proliferate after kidney, lung, liver, or heart injury and give rise to myofibroblasts that contribute to organ fibrosis. Genetic ablation of *Gli1*+ cells significantly reduces fibrosis in multiple organs. In the bone marrow, *Gli1*+ cells line the CD31+ endothelium of bone marrow sinusoids as well as the endosteum of compact bones. Isolated stromal cells derived from *Gli1*+ MSCs express typical MSC markers, including CD44, CD29, CD105, and Sca1, with an absence of CD31, CD45, and CD34.

In summary, research on bone marrow MSCs is transitioning from in vitro analysis based on cell culture, differentiation assays, and surface marker profiling to a greater emphasis on in vivo identification and niche study. These new studies rely on transgenic models to trace and modify mesenchymal cells in mouse models (Table). Studies based on these models have indicated that bone marrow MSCs are perivascular cells in vivo and function as osteogenic stem cells to support bone turnover or injury repair (Shi and Gronthos 2003; Kfoury and Scadden 2015; Mendez-Ferrer et al. 2015). Besides perivascular MSCs, a recent study indicates that Sox9+ or Col2+ chondrocytes within the growth plate, which are not associated with any vasculature, can also contribute to the MSC population and long bone turnover (Ono et al. 2014). This suggests a novel function for chondrocytes, which not only provide the cartilaginous template but also participate in bone formation directly. It remains unclear how much osteogenesis occurs through the chondrocyte pathway.

The neural crest is a population of cells that originates from the dorsal margins of the closing neural folds. These cells then migrate extensively under the induction of signals to various locations in the embryo (Le Douarin and Dupin 2012). Neural crest cells contribute to remarkably diverse tissue types, including the peripheral nervous system, enteric ganglions, cardiac tissue, and the craniofacial skeleton (Le Douarin and Dupin 2012). Based on their origin, neural crest cells can be divided into 4 types: cranial, cardiac, vagal, and trunk (Betancur et al. 2010). The cranial neural crest cells give rise to the majority of the bone and cartilage of the craniofacial region, as well as the nerve ganglia, smooth muscle, connective tissue, and pigment cells. The variety of tissue types to which neural crest cells contribute demonstrates the multipotentiality and self-renewal capacities of these cells, which are cardinal features of stem cells (Stemple and Anderson 1992). Multipotential cranial neural crest cell–derived stem cells have been identified not only from the embryonic tissues but also in adults, a discovery that opens the door for applications of cranial neural crest cell–derived stem cells for regenerative medicine (Zhao et al. 2006; Chung et al. 2009).

Table. Genetic Mouse Models for Studying Adult Bone Marrow Mesenchymal Stem Cells.

Marker	Labeling Method	In Vivo Contribution	Localization	Reference
Nestin	*Nestin-CreERT*	Osteoblasts, osteocytes upon injury repair	Perivascular	Mendez-Ferrer et al. (2010)
LeptinR	*LeptinR-Cre*	Bone, cartilage, adipocytes	Perivascular	Zhou et al. (2014)
Mx1	*Mx1-Cre*	Osteoblasts, osteocytes, hematopoietic stem cells	Perivascular	Park et al. (2012)
Gremlin1	*Gremlin1-CreERT*	Osteoblasts, osteocytes, chondrocytes	Perivascular adjacent to the growth plate and trabecular bone	Worthley et al. (2015)
Gli1	*Gli1-CreERT*	Osteoblasts, osteocytes, chondrocytes, odontoblasts, fibroblasts	Perivascular	Kramann et al. (2015), Zhao et al. (2014)

Dental MSCs

The tooth is composed of enamel, dentin, and soft dental pulp tissue within. The teeth are connected to the alveolar bone through the periodontal ligament (PDL). Human teeth and mouse molars are both brachydont dentitions (low crown, long root). They do not undergo natural turnover and cannot be replaced if lost. Therefore, tissue engineering approaches based on tooth-related stem cells have become the focus of many recent studies.

Dental pulp stem cells (DPSCs) were the first adult stem cells identified from dental tissues (Gronthos et al. 2000). Cells obtained from adult third molars were shown to be highly proliferative and able to undergo osteogenic and chondrogenic differentiation. They also possess adipogenic ability under appropriate conditions, although with a reduced potential. They are positive for classical MSC markers, including CD44, CD73, CD90, CD105, Stro1, and CD146, but are negative for CD34, CD45, and CD14 in vitro. When transplanted into host mice, DPSCs can differentiate into odontoblast-like cells and form dentin-like structure, whereas bone marrow mesenchymal stem cells form distinct bone lamella structure under the same condition (Gronthos et al. 2002).

Subsequently, stem cells were isolated from human deciduous tooth pulp (i.e., SHED [stem cells from human exfoliated deciduous teeth]; Miura et al. 2003) and became more proliferative than bone marrow mesenchymal stem cells or DPSCs. They can undergo trilineage differentiation in vitro and also differentiate into neural cells. They highly express MSC markers, including CD105, CD146, Stro-1, and CD29, but are negative for CD31 and CD34. When transplanted, they form dentin-like structure.

The apical papilla is a transient tissue located at the apex of the root of a developing tooth. It has been proposed to be the cellular source of root formation. Stem cells isolated from the apical papilla (SCAP) have typical MSC properties (Sonoyama et al. 2006; Sonoyama et al. 2008). They can give rise to odontoblasts, osteoblasts, and adipocytes under the proper conditions. They strongly express CD73, CD44, CD105, CD146, and CD166 in vitro. SCAP present a distinct gene expression profile from that of DPSCs. Some markers, such as CD24 and survivin, are strongly expressed in SCAP but not in DPSCs.

The dental follicle is a mesenchymal condensation surrounding the tooth germ during tooth development. Dental follicle stem cells were isolated from dental follicles of the developing third molar (Morsczeck et al. 2005). They can differentiate into osteoblasts, chondrocytes, adipocytes, and neural-like cells in vitro and form cementum and PDL tissue after transplantation into host mice. They are highly positive for CD105, CD44, and CD29 but negative for hematopoietic markers CD34 and CD117 (Vollkommer et al. 2015).

The PDL contains MSCs known as PDLSCs (Seo et al. 2004; Sonoyama et al. 2006). PDLSCs show high expression levels of STRO-1, CD44, CD90, CD105, and CD146. They can differentiate into osteoblasts, chondrocytes, adipocytes, neurons, and even hepatocytes and are therefore multipotential. When transplanted into immunocompromised mice, PDLSCs are able to reconstruct PDL tissues in vivo (Yokoi et al. 2007).

Most of the studies listed above analyzed and defined dental MSCs based on their in vitro properties (Fig. 1). In vitro culture is variable and cannot mimic the stem cell niche. Indeed, some MSC populations defined according to in vitro culture are experimental artifacts (da Silva Meirelles et al. 2008). The identity and regulating mechanisms of dental MSCs in vivo remain largely unknown.

The mouse incisor provides an excellent model for dental MSC study. It grows continuously throughout the lifetime of the animal at a rate of ~365 μm/d, as shown with a tritiated thymidine autoradiography technique (Smith and Warshawsky 1975). Both epithelial and mesenchymal compartments of the incisor rapidly turn over all their cells within 1 mo (Smith and Warshawsky 1975). The continuous turnover of incisor odontoblasts is supported by MSCs in the tooth. As in the long bone, pericytes were first proposed to be the stem cells for the mouse incisor mesenchyme. *NG2* labels pericytes specifically. *NG2*+ pericytes are located surrounding all the vasculatures in the incisor and are immediately adjacent to the endothelium. Through lineage tracing analysis, *NG2*+ pericytes were shown to differentiate into odontoblasts during incisor growth. Upon injury, these *NG2*+ cells also contribute to the formation of the reparative dentin (Feng et al. 2011).

Recent studies identified *Gli1*+ cells surrounding the neurovascular bundle as the stem cells for the incisor dental mesenchyme (Kaukua et al. 2014; Zhao et al. 2014). *Gli1*+ cells are located in the apical region surrounding arterioles but not veins or capillaries. They are normally quiescent and can be activated into proliferation upon injury. Lineage tracing analysis

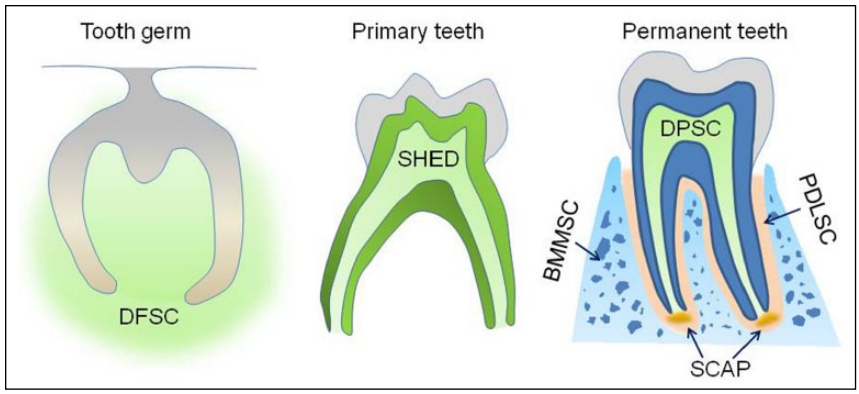

Figure 1. The mesenchymal stem cell populations residing in the tooth–alveolar bone complex. Several mesenchymal stem cell populations have been identified from teeth of different developmental stages and associated alveolar bone, including dental pulp stem cells (DPSC), dental follicle stem cells (DFSC), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSC), stem cells from the apical papilla (SCAP), and bone marrow mesenchymal stem cells (BMMSC).

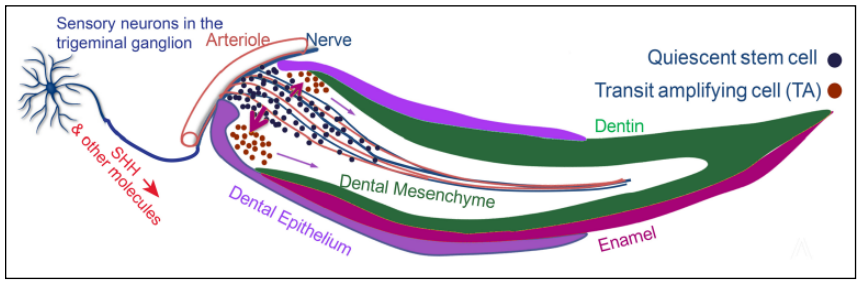

Figure 2. The neurovascular bundle niche and the in vivo origin of incisor mesenchymal stem cells. SHH is secreted by the sensory neurons in the trigeminal ganglion and transported through nerve axons into the incisor mesenchyme. SHH activates *Gli1* expression in the stem cells surrounding the arterioles and regulates their differentiation. *Gli1*+ stem cells give rise to actively dividing transit amplifying cells and then differentiate into odontoblasts to support incisor mesenchyme turnover.

indicated that *Gli1*+ cells give rise to the entire dental mesenchyme in vivo, including the undifferentiated dental mesenchyme, preodontoblasts, and odontoblasts. The majority of *Gli1*+ cells are negative for NG2, CD44, CD146, and other MSC markers in vivo. Cultured incisor mesenchymal cells are typical MSCs according to the classical definition and are almost entirely derived from *Gli1*+ cells. Lineage tracing analysis also indicated that *NG2*+ cells are a subpopulation of *Gli1*+ cells and are derived from them (Fig. 1; Zhao et al. 2014).

Identification of an in vivo marker for incisor MSCs provides the opportunity to study their regulating niche in vivo. *Gli1* expression is an indicator of Hedgehog signaling activity (McMahon et al. 2003). Analysis based on transgenic models and immunohistochemical staining demonstrated that the sensory nerves within the incisor mesenchyme secrete SHH to regulate the incisor MSCs. Denervation abolished *Gli1* activity and caused severe incisor phenotypes. Specific blockage of the Hedgehog signaling pathway also led to differentiation defects of the dentin, suggesting that Hedgehog signaling from the nerve regulates MSC differentiation. The nerves accompany the arterioles to form the neurovascular bundle. This relationship helps to define the location of the *Gli*+ MSCs near their neurovascular bundle niche (Fig. 2; Zhao et al. 2014).

Strikingly, *Gli1*+ cells are absent from adult mouse molars. Mouse molars contain *NG2*+ cells surrounding all types of vasculature. These *NG2*+ cells make no contribution to the dentin under normal physiologic conditions but can contribute significantly to reparative dentin formation upon injury. Mouse molars are similar to human teeth in that neither organ undergoes self-renewal. The absence of *Gli1*+ cells might explain why mouse molars do not have natural turnover (Zhao et al. 2014).

Craniofacial Bone MSCs

Craniofacial bone development is a lengthy process initiated during early embryogenesis and completed during adulthood. Craniofacial bones originate from 2 sources: most are of cranial neural crest origin, but the parietal bones arise from the paraxial mesoderm. Unlike long bones that are connected by well-defined joints, craniofacial bones are connected by sutures. Sutures are the major sites of bone growth during craniofacial development. The expanding brain provides stimulus for the cranial vault to expand (Opperman 2000). The sutures respond by adding bone at the osteogenic front through intramembranous ossification. For sutures to function as growth sites, they need to remain in a patent and unossified state. Previously, it was proposed that the balance among cell proliferation, differentiation, and apoptosis within the sutures is critical for suture patency (Opperman 2000). Craniosynostosis, or the premature closure of ≥1 sutures, can occur when such a balance is disrupted. This condition can lead to craniofacial dysmorphology and other symptoms. Many signaling pathways, such as *Fgf, Bmp, TGF*-β, and *ephrinB*, are critical for the maintenance of suture patency (Slater et al. 2008; Grova et al. 2012; Levi et al. 2012).

Bone marrow MSCs have also been harvested from craniofacial bones, and they exhibit distinct properties from long bone MSCs. Most craniofacial bone marrow MSCs originate from the neural crest cells (Chung et al. 2009), and their gene expression profiles differ from that of long bone MSCs (Matsubara et al. 2005; Fig. 1). Some bone-related congenital diseases affect only the craniofacial bones, such as cherubism (Ueki et al. 2001), Treacher Collins syndrome (Kadakia et al. 2014), craniofacial fibrous dysplasia (Ricalde et al. 2012) and hyperparathyroid jaw tumor syndrome (Pepe et al. 2011),

despite the fact that the genes involved with these diseases are expressed throughout the body. MSCs obtained from craniofacial and long bones also show distinct properties and behavior upon culture and transplantation (Chung et al. 2009). Compared with long bone MSCs, craniofacial bone marrow MSCs proliferate more rapidly, express higher levels of alkaline phosphatase, and form more compact bone and less bone marrow space upon transplantation or culture (Akintoye et al. 2006; Chung et al. 2009). Despite these differences, it was still generally assumed that stem cell regulatory mechanisms and repair mechanisms were similar in craniofacial and long bone MSCs. The repair of a critical-sized calvarial bone defect is routinely used as a standard assay for evaluating the regeneration potential of various types of MSCs.

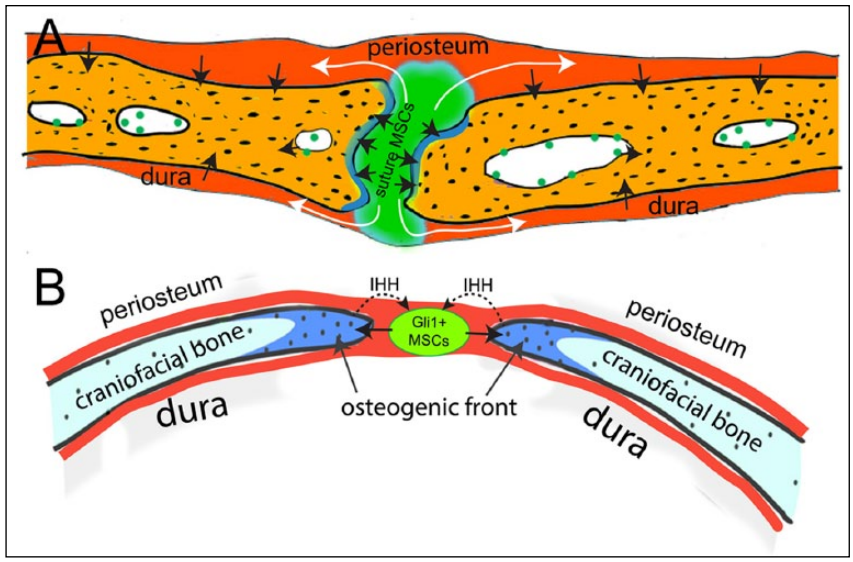

Figure 3. The suture mesenchyme provides a niche for adult craniofacial bone mesenchymal stem cells (MSCs). (**A**) *Gli1*+ MSCs within the suture mesenchyme contribute to the periosteum, osteogenic front, and dura. (**B**) The osteogenic front secretes IHH to regulate the differentiation of *Gli1*+ MSCs in the suture mesenchyme.

The craniofacial periosteum has been proposed to be the source of progenitor cells responsible for injury repair of the adult skull, as it is in long bones (Lin et al. 2014). In long bones, an acute inflammation response is initiated in the periosteum 1 or 2 d after injury. Progenitor cells within the periosteum are then activated into proliferation, causing the periosteum to thicken. A bone callus is formed via endochondral ossification that heals the injury site. Pericytes surrounding the vasculature were proposed to be the progenitor cells within the periosteum (Pape et al. 2010). However, some studies have suggested that the craniofacial periosteum has distinct biological features compared to the long bone periosteum (Lin et al. 2014). When mandibular periosteum is introduced into a tibial bony defect, intramembranous ossification occurs instead of endochondral ossification. If tibial periosteum cells are transplanted into a mandibular defect, endochondral ossification occurs (Leucht et al. 2008).

In addition, a recent study revealed that *Gli1*+ cells are MSCs residing in all craniofacial sutures (Fig. 3; Zhao et al. 2015). These *Gli1*+ cells support the turnover and injury repair of adult craniofacial bones. During postnatal turnover, suture *Gli1*+ cells can give rise to the periosteum and dura (Fig. 2). They are quiescent stem cells and can be activated upon injury. The specific ablation of *Gli1*+ cells leads to the closure of all craniofacial sutures and severe osteoporosis, indicating that they are an indispensable stem cell population. These *Gli1*+ cells are not related to the vasculature, and their distribution is gradually restricted to the suture mesenchyme during postnatal development. Although *Gli1*+ cells do not express typical MSC markers, such as CD44, CD73, or CD146, in vivo, they can undergo trilineage differentiation and highly express all the typical MSC markers after culture in vitro, indicating that they are MSCs. *Gli1*+ cells in the suture mesenchyme are not related to the vasculature and are regulated by IHH secreted from the osteogenic front, which contains committed osteogenic cells (Fig. 3). Blocking the Hedgehog signaling pathway leads to severe osteoporosis, but the sutures remain patent, suggesting that Hedgehog signaling mainly regulates the differentiation, but not maintenance, of these MSCs.

The above study also identified *Gli1*+ cells within the craniofacial bone marrow space. However, their number and function are much less significant than suture *Gli1*+ MSCs based on cellular quantification and transplantation experiments (Zhao et al. 2015). Craniofacial bones contain much less bone marrow space than that of long bones in the adult. Therefore, suture MSCs might be the most important, if not the only, stem cell population for craniofacial bones. The craniofacial periosteum is derived from the suture and therefore is not the most primitive source of stem cells.

These findings provide a new perspective for understanding craniosynostosis. The closure of sutures after stem cell ablation suggests that craniosynostosis might be caused by the premature loss of the stem cell population within the suture mesenchyme. After *Gli1-LacZ* mice were crossed with *Twist1*+/– mice, which are a classical model for studying craniosynostosis (Behr et al. 2011), *Gli1*+ cell number within the sutures was significantly reduced. In addition, although synostosis initiates at around 3 wk after birth in *Twist1*+/– mutants, the number of *Gli1*+ cells was already significantly reduced prior to that, consistent with a causative link between the *Gli1*+ cell reduction and craniosynostosis (Zhao et al. 2015).

Conclusions

Teeth and bones are 2 organs that share many similar developmental and stem cell regulatory mechanisms. Craniofacial bones have typically also been considered similar to long

bones with regard to their repair and stem cell regulation mechanisms. Dental and craniofacial stem cell studies have benefited greatly from earlier MSC and long bone studies. Nevertheless, recent craniofacial studies indicate that these organs are in fact quite different from each other. Craniofacial bones are distinct from long bones, not only in their developmental origins, but also in their stem cell sources and repair mechanisms. This information will have a significant impact on the craniofacial surgery clinic.

In addition, recent MSC studies of mouse incisors and craniofacial bones have challenged the traditional definition of MSCs, which is based on in vitro cellular properties and might not be appropriate for identifying MSCs in vivo. As incisor and suture studies have both shown, the majority of *Gli1*+ MSCs in these organs do not express typical MSC markers, such as CD146 and Sca1. They are unipotential odontogenic or osteogenic stem cells in vivo, even though they can be multipotential in vitro. They are not always perivascular, in contrast to previous proposals that perivascular cells were the in vivo counterparts of MSCs (Feng et al. 2010; Kfoury and Scadden 2015).

The stem cell research field is transitioning from in vitro study to a greater emphasis on in vivo study. Although only a few in vivo models have been established to study stem cells in dental and craniofacial tissues, these studies have already provided valuable information that could not be obtained through in vitro approaches. The lessons learned from these studies will help us to design better strategies for the future use of stem cells for tissue engineering purposes.

Author Contributions

H. Zhao, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Y. Chai, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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