Factors Involved in Mandibular Condylar Growth: An Overview
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Abstract
Although functional appliances have been widely adopted for clinical modulation of mandibular growth for nearly a century, the underlying regulatory mechanism of mandibular condyle growth have been under investigation. Over these years, studies have been focused on both the natural growth of mandibular condyle and its growth modulation by functional appliances at cellular and molecular levels. It has been identified that several factors including Sox9, type II collagen, PTHrP, Ihh, FGFR, type X collagen, VEGF, Cbfa1/Runx2 and OPG/RANKL play a pivotal role in growth and development of mandibular condyle. This review provides an overview of the impacts of these key factors on mandibular condylar growth.

Key Words: Mandibular condylar growth, Regulatory factors, Mandibular advancement

Abbreviations
Sox9: Sex-determining Region on the Y Chromosome (SRY)-related High-Mobility Group (HMG) Box 9; PTHrP: Parathyroid hormone-related protein; Ihh: Indian hedgehog; FGFR: Fibroblast growth factor receptor; VEGF: Vascular endothelial growth factor; Cbfa1: Core binding factor alpha 1; Runx2: Runt-related transcription factor 2; OPG/RANKL: Osteoprotegerin / Receptor activator of NF-kappaB ligand.

Introduction
As functional appliances have been widely used for patients with growth potential, following questions are confronting orthodontists and researchers: What’s the fundamental mechanism of mandibular natural growth and growth modulation? What effects do functional appliances have on mandibular growth? Studies have been conducted on the development of the mandible with or without the use of functional appliance [1-4]. The effectiveness of the bite-jumping appliance in the treatment of Class II malocclusion has been demonstrated by clinical cases [1]. Significant increase in the amount of condylar growth and in the overall length of mandible was observed in animals treated with mandibular forward appliances compared with those in control group [2]. Mandibular protrusion resulted in an increasing number of proliferative cells in the temporomandibular joint, which contributes to the development of the mandibular condyle and glenoid fossa [3]. However, it was claimed by others that functional appliances had no long-term impact on the pattern of mandibular growth although they would be capable of correcting discrepancies in molar relationship and overjet [4]. It has been demonstrated that the effects of functional appliances on mandibular growth can be attributed to independent components including condylar growth and mandibular total rotation [5-7]. This review will discuss the growth and modulation principle of mandible at molecular level from the perspective of condylar growth.

The mandibular condylar cartilage serves as an important growth site of the mandible [8]. It is categorized as the secondary cartilage which is formed during phylogensis and ontogenesis. It is different from the primary cartilage (such as the epiphysal cartilage of long bones) which stems from the organism’s primary cartilaginous skeleton. The growth of primary cartilage is affected by general extrinsic factors, particularly by the growth hormone (STH) and somatomedin [9]. In this sense, orthopedic devices can only change the direction of its growth rather than the amount. On the contrary, the growth of condylar cartilage, the secondary cartilage, is subject to not only general factors like growth hormones but also local factors like mechanical force, thus can be modulated by appropriate orthopedic devices in both the direction and amount of its growth [10]. Several factors have been verified to have great impact on growth and development of mandibular condyle.

Sox9 and type II collagen
Mesenchymal cells in the proliferative layer of developing condyles differentiate into chondrocytes. It has been recognized that the transcription factor Sox9 [Sex-determining Region on the Y Chromosome (SRY)-related High-Mobility Group (HMG) Box 9] plays a key role in chondrocyte differentiation [11]. The function of Sox9 in chondrogenesis was not recognized until it was discovered that mutation in Sox9 could lead to Campomelic Dysplasia (CD), a rare human genetic disease characterized by the hypoplasia of most skeletal components generating from endochondral ossification [12,13]. Sox9 is a transcription factor which consists of two domains: A DNA-binding domain and a transcriptional activator domain. The DNA binding domain contains a High Mobility Group (HMG) box which combines with specific sequence in the minor groove of DNA. Sox9 regulates chondrogenesis in two ways. Firstly, it is essential in the progress that mesenchymal cells differentiate into chondrocytes. It has been shown that, in mouse embryos, the Sox9 gene is expressed in all chondroprogenitor cells and at higher level in chondrocytes during chondrogenesis [14]. In Sox9 null cells of mouse chimeras, there’s no expression of chondrocyte-specific markers [15]. It is also found that misexpression of Sox9 in dermomyotome cells can make these cells deviate from their normal development path towards the cartilage differentiation program [16]. Secondly, Sox9 regulates condylar cartilage formation by modulating the expression of Col2a1 (Core binding factor alpha 1)-encoding type II collagen, the most abundant structural collagen component of cartilage, together with growth potential.
with other elements of cartilage-specific extracellular matrix (collagens IX, XI, the large proteoglycan aggrecan, etc.) [15,17]. Sox9 binds to and activates the 48-bp enhancer element of Col2a1 specific to chondrocytes [18]. If Sox9 protein is truncated with the DNA binding domain retained and the transcriptional activator domain missing, enhancer activity of chondrocytes is shown to be inhibited [18].

Rabie and Hägg found that during natural growth of rats’ condyles, Sox9 was expressed in mesenchymal cells and chondrocytes, indicating its similar effects on regulating mesenchymal cells differentiation and cartilage formation [19]. When the mandible is positioned forward, there is an up-regulation of the expression of Sox9 as well as type II collagen in the condyle and the glenoid fossa, resulting in an increased amount of cartilage formation and subsequent new bone deposition [20,21].

**Parathyroid hormone-related protein (PTHrP)**

There has been a growing interest in the regulatory effects of parathyroid hormone-related protein (PTHrP) on proliferation and differentiation of chondrocytes. The impact of PTHrP deficiency varied greatly between the craniofacial cartilages, according to the distinct features of each cartilage [22].

There was a significant reduction in the number of proliferative chondrocytes in condylar cartilage of homozygous PTHrP-knockout mice [23]. A significant reduction of the hypertrophic-chondrocyte-specific type X collagen was also detected in the PTHrP-knockout group when compared with wild-type group [23].

It is hypothesized that Sox9 is a target for PTHrP signaling [24,25]. PTHrP was observed to up-regulate Sox9, the factor regulating the differentiation of mesenchymal cells into chondroblasts. Therefore, it is suggested that PTHrP may induce the differentiation of mesenchymal cells through the Sox9 pathway. PTHrP may enhance the transcriptional activity of Sox9, facilitating the maintenance of the phenotype of chondrocytes in the pre-hypertrophic zone and hampers their differentiation into hypertrophic chondrocytes [24,25].

It was found that forward positioning of mandible triggered the expression of PTHrP, which led to an increased number of differentiated chondroblasts and meanwhile a retardation of chondrocyte hypertrophy, followed by increased cartilage volume. Therefore, through functional appliance therapy, the condyle is endowed with more potential to construct the cartilage frame for future endochondral bone deposition [26].

**Indian hedgehog (Ihh)**

It has been identified that Indian hedgehog (Ihh), a member of the hedgehog (Hh) family, plays a key role in proliferation, hypertrophy and apoptosis of chondrocytes. A more severe dwarfism was shown in Ihh null mice compared with PTHrP-deficiency mice, suggesting that Ihh acts upon growth plate through pathways both dependent and independent of PTHrP and its receptor [27]. On the one hand, it regulates chondrocytes proliferation in the proliferative zone by independent mechanisms. It has been found that in Ihh-deficiency neonatal mice that condylar growth and organization is hindered due to reduced polymorphic cell layer proliferation and changed fibrocartilaginous nature. Meanwhile, an extremely low level of PTHrP is detected in both the Ihh-deficiency and wild-type group, indicating that Ihh may act directly on progenitor cells by long-range diffusion from its site of origin [28]. This hypothesis has been supported by the observation that there is a strong expression of hedgehog target gene in polymorphic layer [29]. On the other hand, Ihh maintains the pool of proliferative chondrocytes in the growth plate via stimulating PTHrP synthesis by cells in the periarticular perichondrium. Increased signaling through the PTHrP receptor leads to delayed process of chondrocyte hypertrophy and expanded pool of proliferating cells, and vice versa [27]. Studies have demonstrated that in Ihh-deficiency mouse embryos, PTHrP expression and chondrocytes proliferation in mandibular condyle is inhibited [29]. It is recognized that Ihh and PTHrP are key elements of a feedback loop which controls the relative proportions of proliferating and hypertrophic chondrocytes in growth plates [30].

Tang et al. [31] found that mandibular advancement stimulated the expression of Ihh in condylar cartilage resulting in an increased number of proliferating mesenchymal cells and a shortened turnover time. Their findings suggest that Ihh may mediate the conversion process of the mechanical signals caused by mandibular advancement into cellular replication in condylar cartilage [31].

**Fibroblast growth factor (FGF) and its receptors (FGFRs)**

As members of the tyrosine-kinase receptor family, the fibroblast growth factor receptors (FGFRs) are known to have a significant impact on the growth and development of cartilaginous tissues [32].

In mandibular condyle, chondroblasts and hypertrophic chondrocytes showed the most pronounced immunoreactivity for receptors of FGF-2, including FGFR1 and FGFR3. However, immunoreactivity for FGFR2 was found to be strongest in the articular and prechondroblastic zones, indicating that FGF-2 may to some extent, modulate the proliferation activity in condylar cartilage [33].

Designed to create a lateral functional shift of the mandible, intraoral appliances bring about a transverse rotation of the mandible with the condyle on the shifting side remaining in place or moving slightly posteriorly (non-protruded) while the opposite site distracted anteriorly (protruded) from the glenoid fossa. There were significant differences in gene expression for FGF-2, FGFR1, FGFR2 and FGFR3 between the protruded and non-protruded condylar cartilage [34]. The changes in gene expression of FGF-2 and its receptors were suggested to be partly responsible for proliferative activity alterations in condylar cartilage after mandibular shift [34].

**Type X collagen**

Chondrocytes mature after cartilage matrix has been formed. Subsequently, endochondral bone deposition and replacement of cartilage matrix take place. Type X collagen, secreted by hypertrophic chondrocytes, is recognized as an indicator for endochondral ossification and bone replacement of the cartilage matrix [35].

Type X collagen is a homotrimeric molecule which belongs to the short chain collagen family [30]. It has been proved at mRNA and protein levels that the expression of type X collagen is spatiotemporally correlated with endochondral ossification. Spatially, it is detected that type X collagen initially appears throughout the hypertrophic zone when chondrocytes mature, whereas it is missing from the proliferative zone [36]. Therefore, it is considered as a
unique element of the hypertrophic cartilage matrix and a critical marker for endochondral bone formation [30,37]. Temporally, the expression of the type X collagen gene precedes endochondral bone formation [38]. In vitro studies show that its expression takes place before bone deposition by cultured chondrocytes as well [39].

When the mandible is positioned forward by functional appliances, intensive expression of type X collagen is found to distribute throughout the hypertrophic zone when compared with the controls, indicating the onset of condylar endochondral bone formation stimulated by the functional appliances therapy [40]. In addition, Mastication forces also seem to stimulate type X collagen synthesis and bone deposition [41].

**Vascular endothelial growth factor (VEGF)**

Osteogenesis (bone formation) is closely associated with angiogenesis (blood vessels invasion) [42]. The invading blood vessels bring osteogenic progenitor mesenchymal cells into the mineralization front. These cells later differentiate into osteoblasts and engage in osteogenesis. Vascular Endothelial Growth Factor (VEGF) is known to be a potent regulator of neovascularization [42,43].

VEGF is a dimeric glycoprotein, with a molecular mass ranging from 17 to 22 kDa [44]. It is an endothelial cell mitogen and inducer of angiogenesis and microvascular permeability. It was found that VEGF was uniquely produced by hypertrophic chondrocytes immediately before vascular invasion, instead of quiescent or proliferating chondrocytes in avian and mammalian embryo long bones [45].

Both in vivo and in vitro studies show that VEGF receptor 2 can co-localize with the factor in hypertrophic cartilage, which suggests that there may be an autocrine loop in chondrocytes as they mature and hypertrophy. Antibodies against VEGF or VEGF receptor 2 inhibit endothelial cell migration, which indicates VEGF’s critical role in neovascularization of hypertrophic cartilage through a paracrine release targeting at invading endothelial cells [45]. Numerous small blood vessels and vascular structures are found to distribute in the subchondral region where VEGF shows maximal expression [46]. Furthermore, VEGF plays a key role in the replacement of cartilage by bone during growth and repair through angiogenesis. Horner et al. observed co-localized expression of VEGF with alkaline phosphatase in modeling and remodeling sites [47]. When VEGF was inactivated by a soluble receptor chimeric protein systematically administered in mice, blood vessel invasion was almost completely suppressed. Meanwhile, trabecular bone formation is hampered accompanied by expansion of hypertrophic chondrocyte zone [48]. Therefore, it is suggested that VEGF may also play an important role in regulating endochondral ossification in condyles by controlling neovascularization.

Upon forward mandibular positioning by bite-jumping appliance, there was a significant up-regulation of VEGF, followed by accelerated vascularization and subsequently increased new bone formation in both the condyle and glenoid fossa [49,50]. It was found that the temporal expression of VEGF was correlated with the pattern of new bone formation in condyle and glenoid fossa during stepwise mandibular advancement [51,52]. In comparison with one-step advancement, the initial advancement in the stepwise group demonstrated significantly less expression of VEGF. However, VEGF expression appeared to increase significantly in the stepwise group when compared with the single advancement group and the natural growth group. This result suggests that the mechanical stimuli provided by stepwise mandibular advancement may trigger a sequence of cellular events resulting in increased vascularization and bone deposition in condyle [51,52].

**Core binding factor alpha 1 (Cbfal)/ Runt-related transcription factor 2 (Runx2)**

Core binding factor alpha 1 (Cbfal), also known as Runt-related transcription factor 2 (Runx2), has been confirmed to serve as a key regulator of condylar cartilage development, osteoblast differentiation and bone matrix deposition [53-56]. It is demonstrated that Cbfal/Runx2 functions uniquely in the development of mandibular condyle cartilage when compared with in the primary cartilage. Studies have shown that in Runx2-deficient mice, the primary cartilage is not significantly affected while the mandibular cartilage is completely disappeared, suggesting a distinct regulatory effect of Cbfal/Runx2 possibly due to the periosteal origin of condylar cartilage [53,54,56]. Osteoblasts, which function as bone making cells, differentiate from progenitor mesenchymal cells brought by invading blood vessels. Cbfal/Runx2 is an indispensable factor for osteoblast differentiation and bone deposition during endochondral ossification [53,54]. Cbfal/Runx2 is targeted to the promoters of several bone proteins, including osteocalcin (an osteoblast-specific marker), bone sialoprotein, alkaline phosphatase, and type I collagen [53]. It has been shown that in homozygous Cbfal/Runx2-deficient mice, no bone tissue is developed even with the existence of a relatively normal cartilage skeleton [53]. Besides, Cbfal/Runx2 regulates chondrocyte maturation and hypertrophy during endochondral ossification. Rabie et al. found that during the natural development of mandibular condyle, the peaking of Cbfal level coincides with chondrocyte hypertrophy and matrix mineralization [57]. It has also been shown that mandibular advancement triggers Cbfal/Runx2 expression in condylar cartilage, resulting in increased osteoblasts invasion and stimulated chondrocyte terminal maturation [58]. Cbfal/Runx2 is also related to the expression of VEGF and angiogenesis. In homozygous Cbfal null mice, there are no blood vessels growing into cartilage, even if chondrocyte hypertrophy occurs [54]. Overall, Cbfal/Runx2 couples the process of chondrocytes maturation, bone matrix mineralization as well as osteoblasts differentiation during endochondral ossification.

**Osteoprotegerin (OPG) / Receptor activator of NF-kappaB ligand (RANKL)**

It is found that receptor activator of NF-kappaB ligand (RANKL) which belongs to the tumor necrosis factor superfamily together with osteoprotegerin (OPG), its decoy receptor, plays a crucial role in coordinating between osteoblasts and osteoclasts, providing deeper insights into development, turnover and maintenance of bone [59-62]. Osteoclasts carry out the key function of resorbing the mineralized hypertrophic cartilage and facilitating the deposition of osteoblasts to structure the bone matrix. It has been recognized that the ratio of OPG/RANKL plays a major role in the regulation of osteoclastogenesis: an increased ratio
leads to a reduced number of newly formed osteoclasts and vice versa [59]. It was suggested that altered functional loading in temporomandibular joint of mice may lead to an increase in the OPG/RANKL ratio and a subsequent suppression of the formation of osteoclasts, resulting in a return of bone density to the baseline level after a transient decrease [60].

Other studies disclosed that there was an obvious subchondral bone loss following cartilage degradation in rat model with OA (Osteoarthritis)-like lesions in the mandibular condyle. Meanwhile, a decrease of chondrocytes-secreted OPG/RANKL ratio and decrease of OPG level was observed, suggesting its critical role in the osteoclastogenesis of mandibular condyle [61]. However, a more detailed illustration of functional appliances’ effects on endochondral ossification in mandibular condyle achieved by coordination between osteoblasts and osteoclasts still needs to be obtained with the introduction of OPG and RANKL.

**Summary**

A series of events are engaged in the process of condylar growth. Initially, Sox9 transcription factor is expressed in the proliferative layer facilitating the differentiation of mesenchymal cells into chondroblasts. Meanwhile, PTHrP stimulates the differentiation of mesenchymal cells through the Sox9 pathway. FGF and its receptors may also regulate in part the proliferation activity in the mandibular condylar cartilage. Next, chondroblasts synthesize the Type II collagen, marking the onset of cartilage formation. This process is also modulated by Sox9 transcription factor. Chondrocytes proceed to mature and hypertrophy. Hypertrophic chondrocytes generate type X collagen which constitutes the framework of hypertrophic cartilage destined for bone deposition. In the whole process of cartilage formation, Ihh modulates chondrocyte proliferation, hypertrophy and apoptosis through pathways both dependent and independent of PTHrP and its receptor. VEGF is secreted in the hypertrophic cartilage, regulating neovascularization and replacement of cartilage matrix with bone. Bone marrow and resorptive tissue are formed with degradation of hypertrophic cartilage. Osteogenic progenitor mesenchymal cells are brought by invading blood vessels to the cartilage matrix and later differentiate into osteoblasts responsible for osteogenesis. During endochondral ossification, Cbfα1 is essential in regulating maturation of chondrocyte and differentiation of osteoblasts. OPG and RANKL contribute to osteogenesis directly by communicating between osteoblasts and osteoclasts.

It has been proved that mandibular position forwarding by bite-jumping appliance can stimulate mandibular condylar growth by regulating the expression of Sox9, type II collagen, type X collagen at both mRNA and protein levels and VEGF, PTHrP, Ihh at protein level. Further research is expected to explore the gene expressions of these regulatory factors under various circumstances, and to explain the communication between cartilage and bone.

**Conflict of Interests**

There is no conflict of interest.

**References**


