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Deletion of *Runx2* in Condylar Chondrocytes Disrupts TMJ Tissue Homeostasis

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Abstract

Runx2-related transcription factor-2 (*Runx2*) is essential for chondrocyte maturation during cartilage development and embryonic mandibular condylar development. And the process that chondrocytes, especially a subgroup of hypertrophic chondrocytes (HC), could transform into bone cells in mandibular condyle growth makes chondrocytes crucially important for normal endochondral bone formation. To determine whether *Runx2* regulates postnatal condylar cartilage growth and tissue homeostasis, we deleted *Runx2* in chondrocytes in postnatal mice and assessed the consequences on TMJ cartilage growth and remodeling. The cell lineage tracing data provide information demonstrating the role of chondrocytes in subchondral bone remodeling. The histologic and immunohistochemical data showed that *Runx2* deficiency caused condylar tissue disorganization, including loss of hypertrophic chondrocytes and reduced hypertrophic zone, reduced proliferative chondrocytes, and decreased cartilage matrix production. Expression of *Col10a1*, *Mmp13*, *Col2a1*, *Aggrecan* and *Ihh* was significantly reduced in *Runx2* KO mice. The findings of this study demonstrate that *Runx2* is required for chondrocyte proliferation and hypertrophy in TMJ cartilage and postnatal TMJ cartilage growth and homeostasis, and that *Runx2* may play important role in regulation of chondrocyte-derived subchondral bone remodeling.

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AUTHOR CONTRIBUTIONS

Lifan Liao, Shanxing Zhang, Jian Huang and Lan Zhao carried out experiments. Lifan Liao and Di Chen prepared the manuscript. Guang-Qian Zhou, Ling Ye and Lan Zhao helped revising the manuscript. Lan Zhao helped supervising the post-doctoral fellows. Di Chen contributed to the experimental design and data interpretation and finalized the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Keywords

Runx2; temporomandibular joint; chondrocyte; hypertrophy

1 INTRODUCTION

The temporomandibular joint (TMJ) is one of the most frequently used joints in the human body. It consists of the mandibular condyle, the articular disc, the glenoid fossa, and the capsule (Tanaka, Detamore et al. 2008). Animal models including mice and rats are useful to study the development of the TMJ because the process of development/morphogenesis and the molecular mechanisms are well conserved in these animal models (Herring 2003). TMJ condylar cartilage is divided into four zones, including fibrous chondrocyte zone, proliferative chondrocyte zone, mature chondrocyte zone, and hypertrophic chondrocyte zone.

Runx-related transcription factor-2 (Runx2) is a member of the Runt family of transcription factors. During the skeletal development, Runx2 plays a critical role in cellular differentiation processes in osteoblasts and chondrocytes (Lee et al., 1997; Mundlos et al., 1997). Recent studies showed that Runx2 also plays an important role in chondrocyte hypertrophy and OA development (Kamekura et al., 2006; Liao et al., 2017). Despite extensive research on knee joint, less is known about the role of Runx2 in TMJ condylar cartilage development and function.

To explore the role of Runx2 in skeletal development, genetically modified mouse models with *Runx2* deletion or activation have been generated and analyzed, including the global *Runx2* deficiency mice (*Runx2*^{-/-}) (Komori et al, 1997; Otto et al., 1997). Shibata reported that *Runx2*^{-/-} mice lack condylar cartilage, suggesting that Runx2 is essential for the formation of TMJ condylar cartilage (Shibata et al, 2004). However, homozygous *Runx2* global KO mice display stunted growth, low birth weight and die shortly after birth from respiratory insufficiency because of a complete lack of bone tissue (Komori et al, 1997; Otto et al., 1997). Therefore, it's impossible to study the role of Runx2 with homozygous *Runx2* mice at postnatal stage.

Chondrocytes are the only cell type in TMJ cartilage. Moreover, a recent study demonstrated that chondrocytes, especially a subgroup of hypertrophic chondrocytes (HC), could transform into bone cells in mandibular condyle tissue, rather than the old concept of HC apoptosis followed by bone marrow cell invasion (Jing et al. 2015). And this process is responsible for the majority of bone cells, making chondrocytes crucially important for normal endochondral bone formation (Jing et al. 2015). To better understand the function of Runx2 in TMJ cartilage at postnatal stage, it is desirable to delete *Runx2* in a chondrocyte-specific manner. In this study, we generated *Runx2*^{Agc1CreER} mice, in which *Runx2* is specifically deleted in chondrocytes upon tamoxifen administration. Using this mouse model we determined the function of Runx2 in postnatal TMJ cartilage. We found that *Runx2*^{Agc1CreER} KO mice display reduced numbers of proliferative chondrocytes and loss of hypertrophic chondrocytes in TMJ cartilage. These findings provide new evidence that

Runx2 is required for chondrocytes proliferation and hypertrophy in condyle cartilage and maintains TMJ cartilage tissue homeostasis at postnatal stage.

2 MATERIALS AND METHODS

2.1 Animals

ROSA^{mT/mG} reporter mice and *Aggrecan-CreER* (*Agc1-CreER*) transgenic mice were obtained from Jackson laboratories (Bar Harbor, ME, USA). *Runx2^{lox-neo}* mice were provided by Dr. Takeshi Takarada (Takarada et al., 2013) (Okayama University, Japan). *Runx2* floxed mice were generated from the mice carrying a conditional Runx2 allele with exon 4, which encodes the Runt domain, flanked by loxP sites. *Agc1-CreER; ROSA^{mT/mG}* mice and *Agc1-CreER; Runx2^{lox/lox}* (*Runx2^{Agc1CreER}*) conditional KO mice were generated as previously described (Liao et al., 2017). *Agc1-CreER; ROSA^{mT/mG}* mice and *Runx2^{Agc1CreER}* conditional KO mice were administered with tamoxifen (1 mg/10 g body weight/day, i.p. injection, for 5 days) at age 5-week-old and were sacrificed at 9- or 13-week-old for histologic analysis. Cre-negative littermates were used as controls, n=5 per group. Animal protocol of this study has been approved by the IACUC of the Rush University Medical Center and all experimental methods and procedures were carried out in accordance with the approved guidelines.

2.2 Cre-recombination efficiency

To determine whether *Agc1-CreER* mice could target mandibular condylar articular chondrocytes efficiently in adult mice, *Agc1-CreER* transgenic mice were bred with *ROSA^{mT/mG}* reporter mice (Muzumdar et al., 2007) (obtained from Jackson Laboratories). Tamoxifen was administered into 5-week-old mice. Mice were sacrificed at age of 9 weeks. Histologic sections were analyzed using a fluorescence microscope.

2.3 Histology

We dissected skulls from *Runx2^{Agc1CreER}* mice, *Agc1-CreER; ROSA^{mT/mG}* mice and their corresponding Cre-negative control mice. Samples were processed as previously described (Liao et al., 2017) and 3 μ m mid-sagittal sections at 3 different levels (50 μ m apart) were cut from the medial compartment of the TMJ. The sections were stained with Alcian Blue/Hematoxylin-Orange G (AB/HO). 3 slides per mouse, 5 mice per group were analyzed in the experiment.

2.4 Immunohistochemistry

Immunostaining was performed as previously described (Liao et al., 2017). Slides were incubated with primary antibodies against Runx2 (Mouse IgG, MBL, D130-3, 1:200 dilution), Col-X (Rabbit IgG, ab58632, D130-3, 1:1000 dilution), IHH (Rabbit IgG, ab39634, 1:500 dilution), PCNA (Rabbit IgG, ab18197, 1:4000 dilution) at 4°C overnight.

2.5 Real-time PCR analysis

9-week-old *Runx2^{Agc1CreER}* mice and their Cre-negative control mice were sacrificed. TMJ condylar cartilage was collected from freshly sacrificed mice to obtain total RNA. Total

mRNA was extracted with Trizol (Invitrogen Life Technologies, CA). 1 µg total RNA was used to synthesize complementary DNA (cDNA) using an iScript cDNA Synthesis kit (Quanta Biosciences, MD). Real-time PCR amplification was performed using specific primers of genes encoding for cartilage matrix proteins or matrix degradation enzymes and a SYBR Green real-time PCR kit (Quanta Biosciences, MD). The primer names and sequences were listed in Table 1. Data were collected from cartilage of 3 independent mice (n = 3).

2.6 Statistical analysis

Data are presented as the mean $\pm$ SD. For experiments comparing two groups of data, unpaired Student t-test was performed. *P* values less than 0.05 was considered significant.

3 RESULTS

3.1 *Aggrecan-CreER* mice (*Agc1-CreER*) drive Cre recombination in TMJ chondrocytes with high efficiency

To evaluate the Cre recombination efficiency in the TMJ condylar cartilage targeted by *Agc1-CreER* mice, we have bred *Agc1-CreER* transgenic mice with *ROSA^{mT/mG}* reporter mice. *ROSA^{mT/mG}* mice are double-fluorescent Cre reporter mice that express membrane-targeted tandem dimer Tomato (mT) prior to Cre-mediated excision and membrane-targeted green fluorescent protein (mG) after excision. The mG labeling is Cre-dependent (Muzumdar et al. 2007).

Tamoxifen was administrated to mice at ages of 5- (Fig. 1A) and 2-weeks (Fig. 1B) and TMJ samples were harvested at ages of 9- (Fig. 1A) and 4-weeks (Fig. 1B). Analysis of frozen sections of these mice by fluorescence microscopy showed that only red fluorescent image of TMJ condylar cartilage was observed in Cre-negative control mice, indicating that no recombination occurred. In contrast, extensive green-labeled chondrocytes in almost every layer of condylar cartilage were detected in *Agc1-CreER; ROSA^{mT/mG}* mice, indicating that *Agc1-CreER* mice could efficiently target TMJ condylar cartilage cells (Figs. 1A and 1B). Interestingly, we also observed green color in the subchondral bone as well as in the TMJ condylar cartilage (Fig. 1A), indicating the role of the chondrocyte in subchondral bone remodeling.

3.2 Deletion of *Runx2* in chondrocytes at postnatal stage leads to defects in chondrocyte hypertrophy

We then generated *Runx2^{Agc1CreER}* KO mice by breeding *Agc1-CreER* mice with *Runx2^{flax/flax}* mice and investigated the role of Runx2 in TMJ condylar cartilage using the *Runx2* conditional KO mice. Tamoxifen was administrated to mice at ages of 5-weeks and TMJ samples were harvested 4- and 8-weeks after tamoxifen induction when the mice reached 9- and 13-week-old. Serial sections of TMJ condyles were analyzed by histology and histomorphometry. From the superficial cartilage to the deep cartilage of TMJ condyle, chondrocytes in the Cre-negative control mice were well-organized in four layers: the fibrous zone, the proliferative zone, the mature zone, and hypertrophic zone (Figs. 2A and 2B). In contrast, one and two months after *Runx2* deletion at ages of 5 weeks old, condylar zonal organization was disrupted in 9- and 13-week-old *Runx2^{Agc1CreER}* KO mice showing

loss of hypertrophic chondrocytes and reduced hypertrophic zone. In addition, cell loss was also observed in proliferative zone and mature zone (Fig. 3D).

The tidemark in the TMJ condylar cartilage is not obvious in Cre⁻ control mice; however, the tidemark became very obvious at the boundary between the non-calcified and calcified cartilage areas in hypertrophic zone of TMJ condylar cartilage in both 9- and 13-week-old *Runx2^{Agc1CreER}* KO mice (Fig. 2C). Results of AB/HO staining showed that Alcian blue staining intensity was significantly reduced in the TMJ condylar cartilage of *Runx2^{Agc1CreER}* mice, suggesting that proteoglycan production was reduced in *Runx2* KO mice. In Cre⁻ control mice, the Alcian blue staining mainly located on the hypertrophic zone, including non-calcified and calcified areas of TMJ condylar cartilage. In the *Runx2^{Agc1CreER}* KO mice, the Alcian blue staining mainly located on the calcified area of TMJ cartilage adjacent to the subchondral bone (Fig. 2C).

3.3 Changes in chondrocyte differentiation makers in TMJ condylar cartilage of *Runx2^{Agc1CreER}* mice

To assess the efficiency of *Runx2* KO in TMJ condylar cartilage in postnatal *Runx2^{Agc1CreER}* mice, IHC analysis was performed. The results showed that Runx2 protein levels were markedly decreased in TMJ condylar cartilage of *Runx2^{Agc1CreER}* mice compared to the control mice (Fig. 3A). In Cre⁻ control mice, Runx2 expression was detected in proliferative and hypertrophic zones, whereas no Runx2 positive signals in these areas could be detected in TMJ condylar cartilage in *Runx2^{Agc1CreER}* mice (Fig. 3A). Col-X is the most specific marker of hypertrophic chondrocytes and the direct downstream target gene of Runx2. IHC results demonstrated Col-X protein expression were abundant in hypertrophic zone where Runx2 is expressed, while Col-X expression was greatly reduced in *Runx2^{Agc1CreER}* mice (Fig. 3B). IHH is a pre-hypertrophic maker and could promote the proliferation of chondro-progenitor cells. In this study, we found that IHH protein levels are abundant in prehypertrophic zone of TMJ condylar cartilage in Cre-negative control mice, while its expression was greatly decreased in the *Runx2^{Agc1CreER}* KO mice (Fig. 3C). Concurrently, PCNA staining was mainly detected in proliferative chondrocytes of Cre⁻ control mice and was significantly decreased in TMJ condylar cartilage of *Runx2^{Agc1CreER}* KO mice (Fig. 3D), along with the reduction of IHH expression.

3.4 Changes in genes related to cartilage matrix proteins or matrix degradation enzymes in condylar cartilage cells in *Runx2^{Agc1CreER}* KO mice

We then isolated total RNA from TMJ condylar cartilage of 9-week-old Cre-negative control and *Runx2^{Agc1CreER}* KO mice. To assess changes in *Runx2* and genes related to cartilage matrix proteins or matrix degradation enzymes in *Runx2^{Agc1CreER}* KO mice, we performed real-time PCR analysis and examined expression levels of *Runx2*, *Col10a1*, *Mmp13*, *Col2a1*, *Aggrecan* and *Ihh*. The results showed that expression of *Runx2*, *Col10a1*, *Mmp13*, *Col2a1*, *Aggrecan* and *Ihh* was significantly reduced in condylar cartilage cells derived from *Runx2^{Agc1CreER}* KO mice (Figs. 4A–4F), suggesting that expression of anabolic synthesis and chondrocyte differentiation related genes was inhibited in *Runx2* KO cells.

4 DISCUSSION

The TMJ condyle is covered by zonal cartilage layers from the articular surface to the underlying subchondral bone, which serves to absorb shock and contributes to bone remodeling. There are several zonal categorizations of the mandibular cartilage (Silbermann and Frommer, 1972; Durkin et al., 1973; Luder et al., 1988; Piette, 1993; Singh and Detamore, 2009), which result from subtle differences in cell types and ECM contents in the different layers. The classification scheme of four zones reviewed by Singh & Detamore and Wang & Detamore (Wang and Detamore, 2007, Singh and Detamore 2009), consisting of the fibrous, proliferative, mature, and hypertrophic zones, will be mainly referenced in this study. Chondrocytes in upper hypertrophic zone are “typical chondrocytes” that enlarge at a rapid rate, actively produce type II collagen and deposit it into a cartilaginous matrix. The lower hypertrophic zone is composed of chondrocytes at a stage of terminal enlargement while the cartilaginous matrix is adapting for mineralization (Luder et al., 1988). Although multiple references have well reported the role of *Runx2* in knee joint and other long bone, less is known about its role in the development and function of TMJ condylar cartilage.

The cell lineage tracing data in Fig 1 demonstrated the active cell activities (green color) in all layers of TMJ condylar: the surface (progenitor cells), cartilage layer and bone subchondral bone. This result strongly supports the notion that there is a continuous role of endochondral bone remodeling via newly added chondrocytes and bone even at age of 9 weeks, which is consistent with the recent article demonstrating roles of chondrocyte-derived bone cells in condylar formation (Jing et al., 2015).

During cartilage development, *Runx2* controls the maturation and terminal differentiation of hypertrophic chondrocytes, and calcification and vascular invasion during endochondral bone formation (Komori 2005, Lefebvre and Smits 2005, Goldring et al., 2006). In addition, *Runx2* was also reported to influence the pathogenesis of OA through chondrocyte hypertrophy and matrix breakdown after the induction of joint instability (Kamekura et al., 2006). Our previous study showed that deletion of *Runx2* in *Aggrecan-expressing* articular chondrocytes could decelerate the OA progression in adult mice (Liao et al., 2017). These findings suggest the importance of *Runx2* in regulation of chondrocyte hypertrophy.

In the present studies, we found that conditional *Runx2* ablation in postnatal cartilage causes inhibition of chondrocyte hypertrophy and reduced proliferative chondrocytes and decreased cartilage matrix production. Given the severity of these changes, it is clear that continuous *Runx2* expression and action are required for orchestrating and sustaining the chondrocyte proliferation and differentiation during postnatal cartilage growth and maintenance of cartilage homeostasis. Despite the severe loss of hypertrophic chondrocytes in TMJ condylar cartilage in *Runx2* KO mice, the calcified cartilage area is relatively normal, suggesting that the process of chondrocyte terminal differentiation is not totally disturbed. Two possibilities may contribute to this phenotype. One is that the chondrocytes in the calcified area maybe already undergo hypertrophy before tamoxifen administration and *Runx2* deletion. Another possibility is that other *Runx* family numbers may have compensatory effect (Yoshida et al., 2004).

Using IHC assay we found that Runx2 positive signals are mainly located in hypertrophic and calcified area of TMJ condylar cartilage in Cre⁻ control mice, suggesting the role of Runx2 in chondrocyte hypertrophy at the postnatal stage. This finding correlates well with the previous study showing that prominent expression of Runx2 is limited to hypertrophic and post-hypertrophic zones (Ochiai et al., 2010). With the deletion of *Runx2*, protein levels of Col-X, especially in the hypertrophic zone of the TMJ condylar cartilage, significantly decreased in *Runx2^{Agc1CreER}* KO mice compared to Cre⁻ control mice. These findings are consistent with the previous report showing that Runx2 directly controls *Col10a1* transcription through interaction with the cis-enhancing element (Li et al., 2011). MMP13 is a terminal hypertrophic chondrocyte maker (Yang et al., 2014) and is also regulated by Runx2 (Hirata, Kugimiya et al. 2012, Wang, Tang et al. 2012). Interestingly our current study demonstrated the reduced *Mmp13* levels in TMJ condylar cartilage in *Runx2* KO mice.

As demonstrated, Runx2 is highly expressed in both proliferative and hypertrophic chondrocytes and deletion of *Runx2* in chondrocytes at postnatal stage leads to defects in hypertrophic chondrocyte formation in TMJ condylar cartilage. Giving the role of chondrocytes in subchondral bone remodeling (Jing et al., 2015), the information in our article supports the role of Runx2 in regulation of chondrocyte-derived subchondral bone remodeling.

IHH signaling plays a crucial role in postnatal TMJ maintenance for proper tissue homeostasis. Tamoxifen-induced conditional *Ihh* KO mice (*Ihh^{flox/flox}; Col2a1-CreER*) at postnatal days 4, 7, 14, and 56 showed disorganization and growth retardation of condylar cartilage, and reduced chondrocyte proliferation in the TMJ condyle (Ochiai et al., 2010). Consistent with this result, we found concurrent reduction of IHH and PCNA positive cells in the TMJ condylar cartilage after *Runx2* deletion in chondrocytes. In *Ihh*-deficient TMJ condyles, the decreased expression of *Runx2*, *Osterix*, as well as *Col2a1* and *Aggrecan* was observed, suggesting that *Ihh* has the central role in promoting the morphological organization of the condylar cartilage, as well as maintaining the expression of chondrogenic genes (i.e., *Col2a1* and *Aggrecan* etc.) and osteogenic genes (*Runx2* and *Osterix* etc.) (Ochiai et al., 2010). Consistently, in our study, we found that *Runx2* deficiency in chondrocytes led to reduced gene expressions of *Ihh* and chondrogenic genes (*Col2a1* and *Aggrecan*). In this study, we observed that IHH expression is abundant in the mature zone (equal to the pre- hypertrophic zone) and hypertrophic zone of TMJ condylar cartilage in Cre⁻ control mice, while mainly express in less mature chondrocytes in the TMJ condylar cartilage in *Runx2^{Agc1CreER}* mice. With the reduced protein expression of IHH, the proliferative marker PCNA staining was significantly decreased. These observations are consistent with the previous studies demonstrating that Runx2 directly induces *Ihh* expression and co-ordinates the proliferation and differentiation in chondrocytes (Komori, 2005).

Alcian Blue/Hematoxylin-Orange G (AB/HO) stains proteoglycans or glycosaminoglycan (GAG) with blue or purple color in cartilage. The primary proteoglycan in TMJ condylar cartilage is negatively charged aggrecan, capable of binding with a hyaluronic chain to form large aggregates (Poikela et al., 2000). It is known that aggrecan is mainly located in the

hypertrophic and mature zones (Roth et al. 1997, Mao et al. 1998). Consistent with these studies, we found that Alcian blue-positive staining cells are mainly located in hypertrophic zone in TMJ condylar cartilage of Cre⁻ control mice, suggesting hypertrophic chondrocytes are the major cells in TMJ condylar cartilage to produce proteoglycans. While in *Runx2^{Agc1CreER}* KO mice, as the result of loss of hypertrophic chondrocytes, the Alcian blue staining is mainly detected in the calcified area of hypertrophic zone, adjacent to subchondral bone of TMJ.

In summary, in the present studies, we found that deletion of *Runx2* in Aggrecan-expressing chondrocytes causes loss of hypertrophic chondrocytes and decreased proliferative chondrocytes in TMJ condyle. These findings suggest that continuous *Runx2* expression and function is required to orchestrate and sustain the chondrocyte proliferative and hypertrophic processes at the postnatal stage. Giving the role of chondrocytes in subchondral bone remodeling in TMJ condyle, the information from current work also supports the role of *Runx2* in regulation of chondrocyte-derived subchondral bone remodeling.

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Figure 1.

Agc1-CreERT² mice target cells in the condyle and articular eminence areas of temporomandibular joint (TMJ) of *Agc1-CreERT²; ROSA^{mT/mG}* mice. *Agc1-CreERT²; ROSA^{mT/mG}* mice were generated by crossing *Agc1-CreERT²* transgenic mice with *ROSA^{mT/mG}* reporter mice. Tamoxifen (TM, 1 mg/10 g body weight, i.p. injection, x5 days) was administered into 5- (A) and 2-week-old (B) *Agc1-CreERT²; ROSA^{mT/mG}* mice which

were sacrificed 4- (**A**) and 2-weeks (**B**) after TM injection. *Agc1-CreER^{T2}* targeting cells (cells with green color) were detected by fluorescent microscope. DAPI counterstaining was performed. *Agc1-CreER^{T2}* targeting cells (white arrowheads) were detected in condyle, articular eminence and disc by fluorescent microscope. The results showed that high Cre recombination efficiency was observed in TMJ tissues of *Agc1-CreER; ROSA^{mT/mG}* mice. Yellow arrowheads indicate that GFP-positive cells targeted by *Agc1-CreERR^{T2}* are located in cell membrane and cytoplasm (**B**).

Figure 2.

Deletion of *Runx2* in TMJ condyle chondrocytes causes defects in cartilage morphology and chondrocyte hypertrophy. **(A)** Sagittal histologic section of TMJ tissue from 9-week-old Cre-negative control mouse showed four zones of cartilage. From the articular surface to the underlying subchondral bone, the normal TMJ condylar cartilage was divided into fibrous cartilage zone (FZ), proliferative zone (PZ), mature chondrocyte zone (MZ), and hypertrophic zone (HZ). **(B and C)** *Runx2^{Agt1CreER}* conditional knockout (KO) mice

showed significant loss of hypertrophic chondrocytes (cell-free area) and reduced hypertrophic zone in TMJ condylar cartilage. Cre-negative and *Runx2^{Agc1CreER}* mice were administrated with tamoxifen at the age of 5-week-old and TMJ samples were harvested from 9- and 13-week-old mice and Hematoxylin and Eosin (H&E) staining (**B**) and Alcian blue/Hematoxylin-Orange G (AB/HO) staining (**C**) was performed. Histologic results showed that *Runx2^{Agc1CreER}* mice displayed loss of chondrocytes (indicated by blue arrowheads) and reduced hypertrophic zone (the area below the tidemark) in TMJ condylar cartilage. The results of AB/HO staining showed normal hypertrophic chondrocytes (marked by yellow arrowheads in Cre⁻ group) and reduced hypertrophic zone (marked by white arrowheads in *Runx2* KO mice) in TMJ condylar cartilage of *Runx2^{Agc1CreER}* KO mice (n = 5 per group).

Figure 3.

The expression of Runx2 and chondrocyte markers was significantly reduced in *Runx2^{Agc1CreER}* KO mice. Expression of Runx2, Col-X, IHH and PCNA was examined by IHC. (A) The IHC results showed that Runx2 expression was detected in proliferative (red arrowheads) and hypertrophic (blue arrowheads) chondrocytes in *Cre^{-/-}* control mice, while Runx2 expression was undetectable in these regions in *Runx2^{Agc1CreER}* KO mice (right panel). (B) Expression of Col-X was examined and the results showed that expression of Col-X was mainly detected in hypertrophic region in *Cre^{-/-}* control mice (marked by brown arrowheads). In contrast, its expression was significantly decreased in *Runx2^{Agc1CreER}* KO mice (right panel). (C) IHH expression was detected in prehypertrophic region of condylar cartilage in *Cre^{-/-}* control mice (marked by green arrowheads) and was dramatically reduced in TMJ condylar cartilage in *Runx2^{Agc1CreER}* KO mice (right panel). (D) PCNA staining was also performed. PCNA expression was mainly detected in proliferative chondrocytes in *Cre^{-/-}* control mice (marked by yellow arrowheads) and was significantly reduced in *Runx2^{Agc1CreER}* KO mice (right panel).

Figure 4.

Deletion of *Runx2* in chondrocytes leads to decreased expressions of *Col10a1*, *Mmp13*, *Col2a1*, *Aggreacan* and *Ihh* in TMJ condylar cartilage. (A-F) Cre-negative and *Runx2^{Agc1CreER}* mice were administrated tamoxifen at 5-week-old. Total RNA was extracted from TMJ condylar cartilage of 9-week-old Cre-negative control mice (Cre⁻) and *Runx2^{Agc1CreER}* (KO) mice, followed by the real-time PCR assay. Expression of *Runx2*, *Col10a1*, *Mmp13*, *Col2a1*, *Aggreacan* and *Ihh* was significantly reduced in *Runx2* deficient chondrocytes compared to Cre⁻ control mice (* $P < 0.05$ and ** $P < 0.01$, unpaired Student's t-test; $n = 4$ mice per group).

Figure 5.

Cartoon depictions of current findings and the working hypothesis. Loss of *Runx2* in condylar cartilage tissue leads to inhibition of chondrocytes to differentiate into hypertrophic chondrocytes with significantly reduced hypertrophic zone in *Runx2^{Agc1CreER}* conditional knockout (KO) mice. Deletion of *Runx2* also inhibits chondrocytes to translocate into subchondral bone area and transdifferentiate into progenitor cells. FZ = fibrous cartilage zone, PZ = proliferative zone, MZ = mature chondrocyte zone, HZ = hypertrophic zone.

Table 1:

The names and sequences of primers used in this project

Genes	Primer sequence (forward primers)	Primer sequence (reverse primers)
<i>Runx2</i>	GACTGTGGTTACCGTCATGGC	ACTTGGTTTTTCATAACAGCGGA
<i>Mmp9</i>	GCAGAGGCATACTTGTAACG	TGATGTTATGATGGTCCCCTTG
<i>Mmp13</i>	CTTCTTCTTGTTGAGCTGGACTC	CTGTGGAGGTCAGTGTAGACT
<i>Adamts4</i>	ATGGCCTCAATCCATCCCAG	GCAAGCAGGGTTGGAATCTTTG
<i>Adamts5</i>	GGAGCGAGGCCATTTACAAC	CGTAGACAAGGTAGCCCACTTT
<i>Adamts7</i>	GCAGGCTTCGTCTGCTTTCTA	GCCATCAGATAAGGGTTGGTGG
<i>Adamts12</i>	GACCCGAGGCAAGAACATTTT	CCCAGTTGACCGTCAGATTGA
<i>Col10a1</i>	TTCTGCTGCTAATGTTCTTGACC	GGGATGAAGTATTGTGTCTTGGG
<i>Actin</i>	GGCTGTATCCCTCCATCG	CCAGTTGGTAACAAT GCCAT GT