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ABSTRACT

The temporomandibular joint (TMJ) functions as a load-bearing diarthrodial joint during mastication, and its continuous use and stress can lead to degeneration over age. Using senescence-accelerated (SAMP8) mice that develop early osteoarthritis-like changes in synovial joints at high frequency, we analyzed possible molecular mechanisms of TMJ degeneration and tested whether and how malocclusion may accelerate it. Condylar articular cartilage in young SAMP8 mice displayed early-onset osteoarthritic changes that included reductions in superficial/chondroprogenitor cell number, proteoglycan/collagen content, and *Indian hedgehog* (*Ihh*)-expressing chondrocytes. Following malocclusion induced by tooth milling, the SAMP8 condyles became morphologically defective, displayed even lower proteoglycan levels, and underwent abnormal chondrocyte maturation compared with malocclusion-treated condyles in wild-type mice. Malocclusion also induced faster progression of pathologic changes with increasing age in SAMP8 condyles as indicated by decreased PCNA-positive proliferating chondroprogenitors and increased TUNEL-positive apoptotic cells. These changes were accompanied by steeper reductions in *Ihh* signaling and by expression of *matrix metalloproteinase 13* at the chondro-osseous junction in SAMP8 articular cartilage. In sum, we show for the first time that precocious TMJ degeneration in SAMP8 mice is accompanied by—and possibly attributable to—altered *Ihh* signaling and that occlusal dysfunction accelerates progression toward degenerative TMJ disease in this model.

KEY WORDS: superficial cell, osteoarthritis, Indian hedgehog, temporomandibular joint, degenerative joint disease, senescence-accelerated mouse.

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TMJ Degeneration in SAMP8 Mice is Accompanied by Deranged *Ihh* Signaling

INTRODUCTION

The temporomandibular joint (TMJ) is composed of the mandibular condyle, articular disc, and articular eminence/glenoid fossa and functions as a load-bearing and shock-absorbing diarthrodial joint during physiologic activities in vertebrates (Hu *et al.*, 2003; Tanaka *et al.*, 2006). The articular fibrocartilage of the mandibular condyle is referred to as a secondary type of cartilage, and it comprises fibrous tissue and chondrocytes that contribute to its flexibility and elasticity (Shibata *et al.*, 2006). The condylar chondrocytes undergo endochondral ossification and establish a unique cellular organization composed of a fibroblastic superficial layer, a polymorphic layer, a flattened/maturing chondrocyte zone, and a hypertrophic chondrocyte zone (Shibukawa *et al.*, 2007; Wadhwa and Kapila, 2008). The polymorphic layer contains chondroprogenitors exhibiting high mitotic activity and has essential roles for the growth and maintenance of its articular surface (Kantomaa *et al.*, 1994). The flattened zone consists of maturing chondrocytes, while the hypertrophic zone is composed of large-sized chondrocytes undergoing mineralization. The calcified cartilage is ultimately invaded by resorption cavities resulting in replacement of calcified matrix by subchondral bone.

Osteoarthritis in the TMJ is a degenerative structural and functional condition, and its incidence and severity increase with age (Haskin *et al.*, 1995; Moffett *et al.*, 1969). This condition is often accompanied by gradual and progressive loss of proteoglycans, degradation of collagens in the articular cartilage, and sclerosis and increased bone metabolism in subchondral bone resulting in anatomical and functional changes in the condyle (Scrivani *et al.*, 2008; Embree *et al.*, 2011). Studies have examined the incidence of osteoarthritis in TMJ in relation to tooth loss and/or imbalanced occlusion and have concluded that occlusal defects predispose and causally contribute to the disease in both animal models and humans (Hinton and Carlson, 1986; Chen *et al.*, 2009; Wang *et al.*, 2009).

Selective breeding has created several senescence-accelerated (SAMP) mouse strains that display precocious onset and faster progression of age-associated pathologic changes, including excess hair loss, alterations in physical activity, and deficits in learning and memory (Takeda *et al.*, 1981). All the SAMP strains develop degenerative joint disease at different frequency and severity (Hosokawa *et al.*, 1984). Among the SAMP lines, the SAMP8 strain displays early-onset age-related degenerative changes in TMJ's articular surface, including roughness, fissures, and erosion (Chen *et al.*, 1989). The SAMP8 mice have also been used for analysis of experimentally induced condylar osteoarthritis (Chen *et al.*, 1989; Yokoyama *et al.*,

2001). However, little is known about the molecular mechanisms that underlie TMJ degeneration in these experimental models.

Hedgehog proteins constitute a family of secreted signaling molecules that regulate fundamental processes in embryonic and postnatal skeletal development and growth. The proteins act on target cells via the cell surface receptors Patched 1 (Ptc1) and Smoothened (Smo) in cooperation with primary cilia, and signaling is transmitted by Gli zinc-finger transcription factors (Corbit *et al.*, 2005; Haycraft *et al.*, 2005; Koyama *et al.*, 2007; Kinumatsu *et al.*, 2011). In the growth plates of developing endochondral bones including mandibular condyles, Indian hedgehog (Ihh) expression and topography are critical for regulation of chondrocyte and chondroprogenitor proliferation as well as chondrocyte maturation (Koyama *et al.*, 1996; Lanske *et al.*, 1996; St-Jacques *et al.*, 1999; Young *et al.*, 2006; Maeda *et al.*, 2007; Ochiai *et al.*, 2010; Yasuda *et al.*, 2010).

The goal of this study was to identify possible molecular mechanisms underlying TMJ degeneration in senescence-accelerated SAMP8 mice and to ask whether and how malocclusion affects its progression. Our data reveal that early-onset TMJ articular cartilage degeneration in these mice is accompanied by altered Ihh signaling and that occlusal dysfunction accelerates progression toward degenerative morbidity.

MATERIALS & METHODS

Animals

SAMP8/TaSlc (SAMP8) mice were obtained from Japan SLC (Shizuoka, Japan). Animals used in this study were maintained in accordance with the National Institutes of Health's guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee of the Tokyo Dental College. After being placed under general anesthesia with sodium pentobarbital (Somnopenyl, Kyoritsu Seiyaku, Tokyo, Japan) at a dose of 50 mg/kg, 2-mo-old SAMP8 mice were subjected to tooth milling of both upper and lower incisors every other day. Wild-type ($n = 26$), SAMP8 ($n = 8$), tooth-milled wild-type ($n = 12$), and SAMP8 ($n = 21$) mice were maintained on a soft diet and sacrificed by an overdose of anesthetic drug at 2, 2.5, 3, 4, and 6 mo of age.

Histology, Immunohistochemistry, Apoptosis Assay, and *In Situ* Hybridization

TMJs from SAMP8, *Ihh^{fl/fl};Col2 α 1-CreER*, and control mice were fixed with 4% paraformaldehyde overnight, decalcified for 2 wk in 10% EDTA/2% paraformaldehyde, dehydrated, and embedded in paraffin. Serial parasagittal sections of TMJs from operated and control mice were placed on the same slides and processed for histologic, immunohistologic, histochemical, and *in situ* hybridization analyses. Cartilages and bones were stained with Safranin O/Fast Green, Masson's trichrome, and/or hematoxylin and eosin. Apoptosis detection was carried out on paraffin sections with a TUNEL assay kit according to the manufacturer's instructions (Roche, Mannheim, Germany). Fluorescent images were superimposed onto corresponding

bright field images with Adobe Photoshop software. Cell proliferation was carried out with a PCNA staining kit according to the manufacturer's instruction (Invitrogen, Carlsbad, CA, USA). Sections were hybridized with antisense or sense ³⁵S-labeled RNA probes.

Scanning Electron Microscopy

TMJs from mice sacrificed at 6 mo of age were fixed with 4% paraformaldehyde for 20 min at room temperature, and dehydration was achieved by critical-point drying. Dried specimens were coated with gold in a Super Fine Coater (ESC-101, ELIONIX, Tokyo, Japan) and examined with a scanning electron microscope (SU6600, HITACHI, Tokyo, Japan) with 15.0 kV for $\times 70$ and for $\times 1000$.

RESULTS

Abnormal Condylar Organization and Ihh Signaling during SAMP8 TMJ Degeneration

Condyles in 2, 4, and 6 mo-old wild-type mice displayed typical cellular and histologic structure and organization that included a superficial layer, a polymorphic/chondroprogenitor layer, flattened and hypertrophic chondrocyte zones, and subchondral bone (Figure 1A-1C). Proteoglycan levels were low in superficial and polymorphic layers but became higher in the chondrocyte zones, as indicated by Safranin O/Fast Green staining; collagen content was equally high, as indicated by Masson's trichrome staining. *In situ* hybridization showed that the various layers and zones were characterized by expression of typical chondrocyte maturation markers and signaling molecules. Transcripts for *Collagen type I* (*Col I*), a marker of chondroprogenitors and newly differentiated chondrocytes in secondary cartilages (Sugito *et al.*, 2010), were detectable in both the polymorphic layer and the following zone (Figure 2A). *Collagen type II* (*Col II*) was expressed in the upper flattened/hypertrophic chondrocyte zone (Figure 2B), and *Collagen type X* (*Col X*) was expressed in the lower flattened/hypertrophic zone (Figure 2C). *Indian hedgehog* (*Ihh*) transcripts were detected in early hypertrophic chondrocytes (Figure 2D), and Hedgehog transcriptional mediators—*Gli1*, *Gli2*—and Hedgehog transcriptional targets—*Patched* and *Hedgehog-interacting protein*—were detectable in chondroprogenitors and early differentiated chondrocytes (Figures 2E-2H). The condyles from 2-mo-old SAMP8 mice displayed similar overall organization and proteoglycan and collagen content when compared with age-matched wild-type condyles (Figure 1D) but became abnormal with age (Figures 1E, 1F). By 4 and 6 mo of age, the condyles displayed an abnormally thick articular cartilage zone, fewer superficial cells and chondroprogenitors (Figures 1E, 1F, arrowhead), lower territorial and interterritorial proteoglycans/collagen levels, and sparse chondrocyte distribution. While *Col I* and *Col II* transcript levels were slightly reduced (Figures 2I, 2J), those encoding *Ihh*, *Gli1*, *Gli2*, *Ptc1*, *Hip*, and *Col X* were significantly decreased (Figure 2K-2P) indicating that hedgehog signaling and chondrocyte hypertrophy were deranged.

Figure 1. Defective cellular organization and loss of proteoglycan and collagen content in SAMP8 condyles. Sections of mandibular condyles from 2-mo-old (A, D), 4-mo-old (B, E), 6-mo-old (C, F) wild-type (A-C) and SAMP8 (D-F) mice were stained with hematoxylin and eosin (H&E), Safranin O/Fast Green (Saf O/FG) and Masson's trichrome (Mas's). Magnified images are presented of boxed areas. SAMP8 condyles (D) at 2 mo do not display obvious histologic changes compared with wild type (A). Note the significant reduction of superficial and underlying chondroprogenitors in 4- and 6-mo-old SAMP8 articular cartilage (E, F, arrowhead) and the reduction in Safranin O-stained proteoglycans and Masson's trichrome-stained collagens in territorial and interterritorial ECM in 6-mo-SAMP8 articular cartilage (F). *sf*, superficial layer; *pm*, polymorphic layer; *fc/hc*, flattened/hypertrophic chondrocyte zone; *ac*, articular cartilage. Scale bars: 125 µm in low-magnification pictures and 60 µm in high-magnification pictures.

Malocclusion Accelerates Condylar Degeneration in SAMP8 Mice

We next asked whether abnormal mechanical loading caused by malocclusion affected TMJ degeneration in wild-type vs. SAMP8 mice. Malocclusion was experimentally induced by tooth milling at 2 mo of age when the condyles were still anatomically and histologically similar in both mice (Figures 1A, 1D, 3A, 3H). Two weeks after the operation, however, the 2.5-mo-old-SAMP8 condyles displayed abnormal cellular organization and low Safranin O-positive proteoglycans content in superficial and polymorphic layers (Figure 3I, 3J) compared with control condyles from operated wild-type mice (hereafter termed *controls*; Figure 3B, 3C). In superficial cells, the expression of *lubricin*, an important macromolecule for boundary lubrication in synovial joints, was decreased, but there was no

Figure 2. Deranged *Ihh* signaling in SAMP8 condyles. Mandibular condyles from 2-mo-old wild-type (A-H) and SAMP8 (I-P) mice were analyzed by *in situ* hybridization (A-P) with isotope-labeled RNA probes for type I collagen (*Col I*) (A, I), type II collagen (*Col II*) (B, J), type X collagen (*Col X*) (C, K), Indian hedgehog (*Ihh*) (D, L), *Gli1* (E, M), *Gli2* (F, N), *Patched 1* (*Ptch1*) (G, O), and hedgehog interacting protein (*Hip*) (H, P). Note the significant reduction in *Ihh*-positive prehypertrophic chondrocytes and expression in *Gli* transcription factors and Hedgehog transcriptional targets *Ptch1* and *Hip* in SAMP8 articular cartilage. *pm*, polymorphic layer; *fc/hc*, flattened/hypertrophic chondrocyte zone. Scale bars: 125 µm in P for A-P.

apparent change in *Col-II*-expressing differentiating chondrocytes at this stage (Figure 3K, 3L). At 4 wk from the operation, the anterior end of 3-mo-old SAMP8 condyles displayed further loss of proteoglycans (Figure 3M) compared with companion control condyles (Figure 3F, 3G). Interestingly, expression of *Mmp13*, a matrix-degrading molecule, was higher and was particularly so in chondrocytes within the calcified cartilage and the edge of the condyles (Figure 3N, arrowheads).

Figure 3. Degenerative changes are accelerated by malocclusion in SAMP8 condyles. Mandibular condyles from 2-mo-old wild-type (A) and SAMP8 (H) mice exhibit no obvious histologic differences. Similar wild-type (B–G) and SAMP8 (I–N) mice were subjected to incisor tooth milling and sacrificed 2 wk later (2.5-mo overall age) (B–E, I–L) and 4 wk later (3.0 mo) (F, G, M, N). Condyles were analyzed by Safranin O/Fast Green (B, C, F, I, J, M) staining and *in situ* hybridization with isotope-labeled RNA probes for *lubricin* (*lub*) (D, K), *type II collagen* (*Col II*) (E, L), and *Mmp13* (G, N). Boxed areas in B and I are presented with higher magnification in C and J, respectively. Note in 2.5-mo-old operated SAMP8 condyles a deranged cellular arrangement (J, arrowheads), fewer *lubricin*-expressing superficial cells (K), no major changes in *Col II*-expressing chondrocytes (L), and lower Safranin O–stained proteoglycans content in articular surface (M). Note the increased *Mmp13* expression (N, arrowheads) in the anterior region of 3-mo-old SAMP8 condyles compared with wild-type condyles (G). Scale bars: 8 mm in A for A, H; 250 μ m in B for B, I; 65 μ m in C for C, J; and 125 μ m in D for D, E, G, K, L, N.

We asked next whether malocclusion would accelerate progression of TMJ degeneration over age in SAMP8 mice. Malocclusion was experimentally induced by tooth milling at 2 mo of age as above, and TMJs were analyzed at 4 and 6 mo of age. Compared with condyles from mock-treated SAMP8 mice, the condyles from tooth-milled 6-mo-old mice displayed a significant reduction in size along the anteroposterior (longitudinal) and mesio-lateral axes ($p < .02$; Figure 4A, 4F, 4E).

Histologic defects were noted also at the anterior end (Figures 4F, insets, arrowhead), and SEM analysis revealed roughness of articular surface (Figure 4G, insets). Histologic, histochemical, and immunohistologic analyses showed that condylar articular cartilage in operated mice was thinner and there were numerous resorption cavities located directly below it (Figure 4H, arrowhead). Notably, a tidemark indicating the junction between noncalcified and calcified articular cartilage became apparent at 4 mo, and multiple tide marks were observed at 6 mo in tooth-milled mice (Figure 4H, 4I, double arrowhead), indicating abnormal mineralization and chondro-osseous turnover. The condyles also showed a significant and progressive loss of proteoglycan content including aggrecan, compared with condyles from mock-treated SAMP8 mice ($p < .01$; Figure 4C, 4D, 4H–4J). *In situ* hybridization on condyles from 4-mo-old tooth-milled SAMP8 mice showed decreased expression of *Ptch1* in chondroprogenitors (Figure 4M) indicating disruption of *Ihh* signaling. Interestingly, expression of *Mmp13* was higher in chondrocytes within the calcified cartilage compared with mock-treated mice (Figure 4L, 4N). To verify that deranged *Ihh* signaling is involved in reduction of condylar cartilage area, condyles from conditional *Ihh^{fl/fl};Col2 α 1-CreER* mutant mice were processed for histology and *in situ* hybridization. Indeed, mutant condylar cartilage was thinner and displayed a substantial decrease in *Aggrecan* expression (Appendix Figure 1).

Decreased Chondroprogenitor Cell Proliferation and Increased Cell Death in SAMP8 Condyles after Malocclusion

To identify possible cellular mechanisms underlying changes described above, condyles from mock-treated and tooth-milled SAMP8 mice were isolated at 2 wk after tooth milling (when the mice were 2.5 mo old) and processed for cell proliferation and cell apoptosis analyses. Several PCNA-positive proliferating cells were present in the polymorphic layer in wild-type and mock-treated SAMP8 condyles, although far fewer were seen in tooth-milled SAMP8 condyles (Figure 4O, 4Q, 4S; Appendix Figure 2). There were more TUNEL-positive cells in the polymorphic cell and maturing chondrocyte zones (arrows) in tooth-milled SAMP8 mice than mock-treated SAMP8 and wild-type mice (Figure 4P, 4R, 4T; Appendix Figure 2).

DISCUSSION

Our results show that both thickening and thinning of condylar cartilages are associated with attenuated *Ihh* signaling activity, as shown by decreased expression of *Gli1*, *Gli2*, and/or *Ptch1* in *Ihh* target cells. The increased cartilage thickness present in SAMP8 condylar cartilages at 4 and 6 mo of age is an unexpected observation. We do not have yet a clear understanding and explanation of the underlying cellular and molecular mechanisms. We observe decreased proliferation in chondroprogenitors and decreased synthesis of proteoglycans/collagens, and there is a sparse chondrocyte distribution in the cartilage matrix. It is thus safe to propose that deranged *Ihh* signaling would indirectly elicit a thickening of condylar cartilage through abnormal matrix turnover and accumulation. The thinning of

Figure 4. Degenerative changes are accelerated by malocclusion in SAMP8 condyles. Two-month-old SAMP8 mice subdivided into control mock-milled (A-D, K, L, Q, R) and tooth-milled (F-I, M, N, S, T) groups and sacrificed at 2.5 mo (Q-T), 4 mo (C, H, K-N), and 6 mo of age (A, B, D, F, G, I). Condyles were analyzed by macroscopy (A, F), SEM (B, G), hematoxylin and eosin staining (H&E) (C, D, H, I; left panel), Safranin O/Fast Green staining (Saf O/FG) (C, H; right panel), aggrecan immunohistochemistry (D, I; right panel), and *in situ* hybridization with isotope-labeled probes for *Ptch1* (K, M) and *Mmp13* (L, N). Note the flattening of the anterior end of the mandibular condyle in the tooth-milled group compared with controls (A, F, respectively). Note that the malocclusion-treated condyle is smaller along the anterior-posterior and mesolateral axes and that there is roughness of the articular surface compared with controls (B, G, respectively). Note also in malocclusion-subjected SAMP8 condyles that there is lower Safranin O staining (H), multiple tidemarks, and presence of resorption cavities (H, I, double arrowheads and arrowhead, respectively). Statistical analyses confirm changes of condylar size ($*p < .02$, $n = 4$ for each group) and proteoglycan content ($**p < .01$, $n = 7$ for each group) (E, J). Results are expressed as Safranin O-positive area/entire cartilage field obtained by 40 $\times$ objectives (mean $\pm$ SD, $n = 7$ for each group). Note the reduced *Ptch1* expression and increased *Mmp13* expression. Serial parasagittal sections from 2.5-mo-old wild-type (O, P), mock- (Q, R), and malocclusion-treated (S, T) SAMP8 condyles were processed for PCNA (O, Q, S) and TUNEL staining (P, R, T). Data were collected from randomly selected 7 sections (approximately 120-150 cells/area) per sample and presented as averages $\pm$ SD; p values $< .05$ were considered statistically significant ($*p < .05$, $**p < .02$). Note the significant decrease in proliferating chondroprogenitor cells in superficial/polymorphic layers and increased apoptosis in chondroprogenitors and chondrocytes in malocclusion-subjected SAMP8 condyles. Schematic depicting our current working model of early-onset degenerative TMJ disease in SAMP8 mice and its acceleration by malocclusion. Scale bars: 1.8 mm in A for A, F; 0.7 mm in B for B, G; 80 μ m in C for C, D, H, I; 200 μ m in K for K-N; and 40 μ m in P for O-T.

condylar cartilage that we observe in SAMP8 mice occlusally challenged with tooth milling probably has a different origin. Given that thinning occurs also after conditional *Ihh* deletion in cartilage, it is likely that it may be due to further attenuation of *Ihh* signaling. Cartilage thinning is accompanied by further decreases of chondroprogenitors' mitotic activity, further increase of chondrocyte apoptosis, and ectopic expression of catabolic enzymes, indicating that tooth-milled SAMP8 condyles are very susceptible to abnormal loading and progress toward a pathologic condition. Additional comparisons of catabolic pathways and processes in *Ihh* mutants vs. SAMP8 tooth-milled mice will provide further mechanistic insights into condylar cartilage phenotypes.

It is important to take into consideration indirect mechanisms that could likely contribute to decrease *Ihh* signaling in SAMP8 condyles. For instance, growth defects in mandibular condyles observed in response to experimentally induced mechanical stress were recently proposed to be due to changes in signaling and action by BMPs, VEGF, and PTHrP/PTH (Owtad *et al.*, 2013). We have also shown that constitutive FGFR signaling altered endochondral ossification in the mandibular condyles of *FgfR3^{P244R}* mutant mice, likely by reducing hedgehog signaling (Yasuda *et al.*, 2012). Thus, further experiments, including crossing the SAMP8 mice with *Ihh* mutants, will be necessary to test and clarify the possible genetic interactions among these pathways.

TMJ's articular surface has been shown to possess a remarkable adaptive potential in response to mechanical force, and the aging process may lower such capacity (Mongini, 1980; Bouvier, 1988). We document here that the organization and cell density of articular cartilage change precociously in SAMP8 mice over age and that these changes are significantly accelerated by mechanical stress caused by tooth milling. Compared with age-matched wild-type TMJs, the SAMP8 TMJs show earlier pathogenic changes in periarticular tissues, including locally deranged cellular organization and decreased *lubricin*-expressing superficial cells. The destructive changes are particularly severe at the anterior end of the condyles of tooth-milled SAMP8 TMJs and are accompanied by increased ectopic expression of matrix-degrading enzymes. Our data suggest that the periarticular tissues of condyles may represent the primary degenerative tissue sites undergoing initial wear and tear. Progression of osteoarthritis often leads to morphologic changes in the joints due to ectopic cartilage/bone formation (osteophytes), and studies have shown that there is a positive correlation between increases in hedgehog signaling and pathologic changes of degenerative articular cartilage (Lin *et al.*, 2009). The lack of ectopic cartilage/bone formation in the TMJs of SAMP8 mice that we observe at 4 mo post-tooth milling may be due to their low *Ihh* signaling and reduced hedgehog-responding periarticular cells. Therefore, ectopic activation and/or early reduction of *Ihh* signaling could significantly alter TMJ responses and affect the progression of articular chondrocytes and the joints themselves toward disease states.

In summary, our study reveals that a downregulation of *Ihh* signaling accompanies the early onset TMJ degenerative changes in senescence-accelerated mice, suggesting that such

decrease—combined with abnormal mechanical loading—could accelerate progression of TMJ pathology. The evidence presented here suggests possible future therapies in which an early restoration of hedgehog signaling could minimize or even prevent TMJ degeneration and could restore function, particularly in cases when malocclusion is a factor.

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REFERENCES

- Bouvier M (1988). Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. *J Dent Res* 67:1206-1212.
- Chen J, Sorensen KP, Gupta T, Kilts T, Young M, Wadhwa S (2009). Altered functional loading causes differential effects in the subchondral bone and condylar cartilage in the temporomandibular joint from young mice. *Osteoarthritis Cartilage* 17:354-361.
- Chen WH, Hosokawa M, Tsuboyama T, Ono T, Iizuka T, Takeda T (1989). Age-related changes in the temporomandibular joint of the senescence accelerated mouse: SAM-P/3 as a new murine model of degenerative joint disease. *Am J Pathol* 135:379-385.
- Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF (2005). Vertebrate Smoothed functions at the primary cilium. *Nature* 437:1018-1021.
- Embre M, Ono M, Kilts T, Walker D, Langguth J, Mao J, *et al.* (2011). Role of subchondral bone during early-stage experimental TMJ osteoarthritis. *J Dent Res* 90:1331-1338.
- Haskin CL, Milam SB, Cameron IL (1995). Pathogenesis of degenerative joint disease in the human temporomandibular joint. *Crit Rev Oral Biol Med* 6:248-277.
- Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK (2005). Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet* 1:e53.
- Hinton RJ, Carlson DS (1986). Response of the mandibular joint to loss of incisal function in the rat. *Acta Anat (Basel)* 125:145-151.
- Hosokawa M, Kasai R, Higuchi K, Takeshita S, Shimizu K, Hamamoto H, *et al.* (1984). Grading score system: a method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). *Mech Ageing Dev* 26:91-102.
- Hu K, Qiguo R, Fang J, Mao JJ (2003). Effects of condylar fibrocartilage on the biomechanical loading of the human temporomandibular joint in a three-dimensional, nonlinear finite element model. *Med Eng Phys* 25:107-113.
- Kantomaa T, Tuominen M, Pirttiniemi P (1994). Effect of mechanical forces on chondrocyte maturation and differentiation in the mandibular condyle of the rat. *J Dent Res* 73:1150-1156.
- Kinumatsu T, Shibukawa Y, Yasuda T, Nagayama M, Yamada S, Serra R, *et al.* (2011). TMJ development and growth require primary cilia function. *J Dent Res* 90:988-994.
- Koyama E, Leatherman JL, Noji S, Pacifici M (1996). Early chick limb cartilaginous elements possess polarizing activity and express hedgehog-related morphogenetic factors. *Dev Dyn* 207:344-354.

- Koyama E, Young B, Nagayama M, Shibukawa Y, Enomoto-Iwamoto M, Iwamoto M, *et al.* (2007). Conditional Kif3a ablation causes abnormal hedgehog signaling topography, growth plate dysfunction, and excessive bone and cartilage formation during mouse skeletogenesis. *Development* 134:2159-2169.
- Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, *et al.* (1996). PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science* 273:663-666.
- Lin AC, Seeto BL, Bartoszko JM, Khoury MA, Whetstone H, Ho L, *et al.* (2009). Modulating hedgehog signaling can attenuate the severity of osteoarthritis. *Nat Med* 15:1421-1425.
- Maeda Y, Nakamura E, Nguyen MT, Suva LJ, Swain FL, Razzaque MS, *et al.* (2007). Indian hedgehog produced by postnatal chondrocytes is essential for maintaining a growth plate and trabecular bone. *Proc Natl Acad Sci U S A* 104:6382-6387.
- Mongini F (1980). Condylar remodeling after occlusal therapy. *J Prosthet Dent* 43:568-577.
- Moffett BC Jr, Johnson LC, McCabe JB, Askew HC (1969). [The articular reconstruction of the temporomandibular joint in the adult]. *Minerva Stomatol* 18:611-630. Article in Italian.
- Ochiai T, Shibukawa Y, Nagayama M, Mundy C, Yasuda T, Okabe T, *et al.* (2010). Indian hedgehog roles in post-natal TMJ development and organization. *J Dent Res* 89:349-354.
- Owtad P, Park JH, Shen G, Potres Z, Darendeliler MA (2013). The biology of TMJ growth modification: a review. *J Dent Res* 92:315-321.
- Scrivani SJ, Keith DA, Kaban LB (2008). Temporomandibular disorders. *N Engl J Med* 359:2693-2705.
- Shibata S, Suda N, Suzuki S, Fukuoka H, Yamashita Y (2006). An in situ hybridization study of Runx2, Osterix, and Sox9 at the onset of condylar cartilage formation in fetal mouse mandible. *J Anat* 208:169-177.
- Shibukawa Y, Young B, Wu C, Yamada S, Long F, Pacifici M, *et al.* (2007). Temporomandibular joint formation and condyle growth require Indian hedgehog signaling. *Dev Dyn* 236:426-434.
- St-Jacques B, Hammerschmidt M, McMahon AP (1999). Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev* 13:2072-2086.
- Sugito H, Shibukawa Y, Kinumatsu T, Yasuda T, Nagayama M, Yamada S, *et al.* (2010). Ihh signaling regulates mandibular symphysis development and growth. *J Dent Res* 90:625-631.
- Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, *et al.* (1981). A new murine model of accelerated senescence. *Mech Ageing Dev* 17:183-194.
- Tanaka E, Yamano E, Dalla-Bona DA, Watanabe M, Inubushi T, Shirakura M, *et al.* (2006). Dynamic compressive properties of the mandibular condylar cartilage. *J Dent Res* 85:571-575.
- Wadhwa S, Kapila S (2008). TMJ disorders: future innovations in diagnostics and therapeutics. *J Dent Educ* 72:930-947.
- Wang MQ, Xue F, He JJ, Chen JH, Chen CS, Raustia A (2009). Missing posterior teeth and risk of temporomandibular disorders. *J Dent Res* 88:942-945.
- Yasuda T, Mundy C, Kinumatsu T, Shibukawa Y, Shibutani T, Grobe K, *et al.* (2010). Sulfotransferase Ndst1 is needed for mandibular and TMJ development. *J Dent Res* 89:1111-1116.
- Yasuda T, Nah HD, Laurita J, Kinumatsu T, Shibukawa T, Shibutani T, *et al.* (2012). Muenke syndrome mutation, *FgfR3*^{P244R}, causes TMJ defects. *J Dent Res* 91:683-689.
- Yokoyama T, Murakami H, Hozumi E, Kato D, Ito Y, Maeda H, Kameyama Y. (2001). Effect of the mandibular condyle in senescence-accelerated mouse losing occlusion: Histopathological and histometrical evaluation. *J Jpn Soc.* 13:211-218.
- Young B, Minugh-Purvis N, Shimo T, St-Jacques B, Iwamoto M, Enomoto-Iwamoto M, *et al.* (2006). Indian and sonic hedgehogs regulate synchondrosis growth plate and cranial base development and function. *Dev Biol* 299:272-282.