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ABSTRACT

Primary cilia regulate limb and axial skeletal formation and hedgehog signaling, but their roles in temporomandibular joint (TMJ) development are unknown. Thus, we created conditional mouse mutants deficient in ciliary transport protein Kif3a in cartilage. In post-natal wild-type mice, primary cilia were occasionally observed on the superior, inferior, or lateral side of condylar cells. Cilia were barely detectable in mutant chondrocytes but were evident in surrounding tissues, attesting to the specificity of chondrocyte *Kif3a* ablation. Mutant condyles from 3-month-old mice were narrow and flat along their antero-posterior and medio-lateral axes, were often fused with the articular disc, and displayed an irregular bony surface. The polymorphic layer in P15 mutants contained fewer *Sox9*-expressing chondroprogenitor cells because of reduced mitotic activity, and newly differentiated chondrocytes underwent precocious hypertrophic enlargement accompanied by early activation of *Indian hedgehog* (*Ihh*). Interestingly, there was excessive intramembranous ossification along the perichondrium, accompanied by local expression of the hedgehog receptor *Patched-1* and up-regulation of *Osterix* and *Collagen I*. In summary, Kif3a and primary cilia are required for coordination of chondrocyte maturation, intramembranous bone formation, and chondrogenic condylar growth. Defects in these processes in *Kif3a* condylar cartilage are likely to reflect abnormal hedgehog signaling topography and dysfunction.

KEY WORDS: TMJ, mandibular condyle, primary cilia, Kif3a, hedgehog signaling.

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TMJ Development and Growth Require Primary Cilia Function

INTRODUCTION

The mandibular condyle in vertebrates functions as a diarthrodial joint with the glenoid fossa and disc and as an engine for mandibular growth. In early embryogenesis, condyle formation initiates with a thickening of periosteal tissues along the jaw anlagen. Condylar chondrocytes then become organized in a growth-plate-like structure that displays 4 distinct zones along its main axis: a fibrous superficial layer, a polymorphic chondroprogenitor layer, a flattened chondrocyte zone, and a hypertrophic chondrocyte zone (Luder *et al.*, 1988; Shibukawa *et al.*, 2007). The chondroprogenitor cells proliferate and differentiate into chondrocytes that are apposed to the underlying condylar cartilage, thus sustaining elongation (Kantomaa *et al.*, 1994). The chondrocytes then undergo hypertrophy, which further contributes to growth and allows for the replacement of cartilage with endochondral bone and marrow. Concomitantly, intramembranous bone develops around the periphery of hypertrophic chondrocytes to form a collar (Koyama *et al.*, 1996). All these processes are as well-orchestrated and -coordinated as those occurring in developing long bones, but interestingly, the condylar chondrocytes are not as well organized in columns (Shibata *et al.*, 2006).

Indian hedgehog (*Ihh*) is a major regulator of pre- and post-natal long bone development (Nakamura *et al.*, 1997; St-Jacques *et al.*, 1999; Maeda *et al.*, 2007) and is also critical for condylar development, as has been shown previously (Shibukawa *et al.*, 2007; Ochiai *et al.*, 2010; Yasuda *et al.*, 2010). Hedgehog proteins act on target cells by interaction with the cell-surface receptor Patched (Ptch1) and activation of Smoothened. Signaling is transmitted by processing of zinc-finger transcription factors Gli1, Gli2, and Gli3 in primary cilia into transcriptional activators and repressors (Lum and Beachy, 2004; Huangfu and Anderson, 2006; Haycraft *et al.*, 2007). Primary cilia are solitary, immotile, and microtubule-based organelles that project from the surfaces of most vertebrate cells, including chondrocytes (Scherft and Daems, 1967; Davenport and Yoder, 2005; Donnelly *et al.*, 2008; Serra, 2008). The process of intraflagellar transport (IFT) is responsible for building and maintaining the structure of primary cilia. Mutations in structural and functional components of primary cilia cause defects in patterning, skeletal development, and other processes (Haycraft *et al.*, 2005, 2007; Song *et al.*, 2007). For example, ablation of the kinesin II-motor complex component Kif3a or the ciliary structural component Polaris disrupts not only ciliary organization, but also cranial base synchondrosis and long bone development. The growth plates in these structures were totally disorganized and displayed minimal

chondrocyte proliferation and persistence of hypertrophic cells (Haycraft *et al.*, 2007; Koyama *et al.*, 2007; Song *et al.*, 2007; Ochiai *et al.*, 2009). *Ihh* signaling was deranged and accompanied by a drastic reduction in expression of *Ptch1* and *Gli1*, which are direct transcriptional targets of Hedgehog (Hh) signaling in both growth plate chondrocytes and surrounding perichondrium.

We conducted the present study to determine whether primary cilia have similar essential roles in TMJ development, morphogenesis, and growth. Given the rather unique structural and developmental features of the TMJ, we were particularly interested in clarifying whether defects in primary cilia in chondrocytes would alter the endochondral and intramembranous bone formation processes and lead to mandibular condylar defects.

MATERIALS & METHODS

Generation of *Col2a-Cre*; *Kif3a* Conditional Mutant Mice

The generation of *Kif3a^{fl/fl}*; *Col2a1-Cre* mice was described previously (Song *et al.*, 2007). Animals used in this study were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at our respective Institutions.

Micro-computed Tomography

Skulls fixed in buffered 4% paraformaldehyde overnight at 4°C were subjected to micro-computed tomography (μ CT) with a CT40 SCANCO Medical System (Southeastern, PA, USA) with a 36-mm holder at 45 kV of energy, 12- μ m scanning thickness, and medium resolution. Two-dimensional slice images were selected and used to generate three-dimensional reconstructions with the following parameters: filter width sigma = 0.8, support level = 1.0, and threshold = 173. The same values were used to analyze wild-type and mutant samples at each specified time-point. Three-dimensional images were rotated at specific angles to generate a lateral view of the mandibles and condyles.

Skeletal Preparation

Mouse heads were dissected and fixed with 95% EtOH for 24 hrs at room temperature. After removal of soft tissues, specimens were stained with alizarin red (Sigma, St. Louis, MO, USA) for 3 days at 37°C, cleared in 1% KOH, and stored in 80% glycerol. Mandibles were photographed and measured macroscopically.

Histology, Immunohistochemistry, and Gene Expression Analysis

Kif3a mutants and control littermates were fixed with 4% paraformaldehyde overnight, decalcified for 2 wks by 10% EDTA/2% paraformaldehyde, and embedded in paraffin. Serial frontal and/or parasagittal sections from mutants and control littermates, placed on the same slides, were processed for histology, immunohistochemistry, and *in situ* hybridization (Koyama *et al.*, 2007). In total, 24 wild-type mice, including 4 pre-natal

embryos, 6 newborns, 5 one-week-old, 6 two-week-old, and 3 three-month-old mice, and 16 *Kif3a*-conditional mutants, including 3 pre-natal embryos, 4 newborns, 3 one-week-old, 3 two-week-old, and 3 three-month-old mice, were used. Cartilage and bones were stained with Safranin-O/fast green. For immunofluorescence, 10- μ m-thick sections were treated with 0.1% trypsin for 10 min at room temperature, and incubated with 1:200 dilution of acetylated α -tubulin monoclonal antibody (Clone 6-11B-1: Sigma, St. Louis, MO, USA), followed by 1:200 dilution of AlexaFluor568-conjugated anti-mouse IgG (Invitrogen, Carlsbad, CA, USA) and DAPI (Invitrogen). Sections were hybridized with antisense or sense ³⁵S-labeled probes (Nagayama *et al.*, 2008). Mouse cDNA clones were: *histone 4C* (nt. 549-799; AY158963); *Sox9* (nt. 116-856; NM_011448); *collagen I* (nt. 233-634; NM_007742); *collagen II* (nt. 1095-1344; X57982); *collagen X* (nt. 1302-1816; NM_009925); *Osterix* (nt. 40-1727; NM_130458); *Ihh* (nt. 897-1954; MN_010544); *lubricin* (nt. 41-2646; AB034730); *Fibromodulin* (nt. 2023-2583; NM_021355); and *Patched-1* (nt. 81-841; NM_008957).

RESULTS

TMJ Defects in Conditional *Kif3a*-deficient Mice

We created mice deficient in *Kif3a* in cartilage by mating floxed *Kif3a* with *Col2a1-Cre* mice, and the resulting TMJs from *Kif3a^{fl/fl}*; *Col2a1-Cre* mice (heretofore termed '*Kif3a*-deficient') and *Kif3a^{fl/+}*; *Col2a1-Cre* and *Kif3a^{fl/fl}* mice (heretofore termed 'controls') were analyzed. To gain overall insights, we first processed the mandibles from 3-month-old control and *Kif3a*-deficient mice for μ CT analysis and whole-mount alizarin red staining. Compared with controls, *Kif3a*-deficient mandibular bones were slightly hypomorphic and characterized by extremely elongated lower incisors (Fig. 1G), possibly due to severe developmental defects in cranial base synchondroses (Koyama *et al.*, 2007; Ochiai *et al.*, 2010) that could have led to malocclusion against upper incisors by 4 to 6 wks of age. Mutant condylar bony surfaces were irregular (Fig. 1H, arrowhead), the subchondral bone consistently contained fewer trabeculae (Appendix Fig. 1), and articular cartilage was thinner (Fig. 1K, brackets) compared with controls (Figs. 1B, 1E, brackets). A superior view revealed that the condyles were narrower along their medio-lateral axis, were flatter along their antero-posterior axis (Fig. 1I, horizontal and vertical double arrows, respectively), and had anterior defects (Figs. 1H, 1I, double arrowheads). The narrow medio-lateral axis of the mutant condyle was clear when viewed from the back (Fig. 1J), and the articular disk was actually fused with the condyle itself (Fig. 1K, arrowhead). In addition, mutant mandibular symphysis, where secondary cartilage formation takes place (Sugito *et al.*, 2011), was defective.

Primary Cilia Analysis

In control condyles, primary cilia in superficial/polymorphic (sf/pm) cells were occasionally observed in the superior, inferior, or lateral side of the cells (Figs. 2A, 2B). Cilia were barely detectable on mutant condylar chondrocytes (Figs. 2G, 2H), but were

Figure 1. Mandibular bone, symphysis, and condylar development are defective in *Kif3a*-deficient mice. Mandibles from 3-month-old control (A-F) and *Kif3a*-deficient (G-L) mice were analyzed by micro-computed tomography (A-B, F, G-H, I), macroscopy (C-D, J), and histology (E, K). Highly magnified sagittal view of condylar surface (H, arrowhead) displays irregularities of its bony surface compared with that of wild-type mice (B, arrowhead). Superior (C, I) and back (D, J) views of condyles. Note that lengths along antero-posterior and medio-lateral axes (C, I, horizontal and vertical double arrows, respectively) are shorter in *Kif3a*-deficient than in control mice and that the most anterior portion of the condyle is defective as well (B, C, H, I, double arrowheads). Safranin-O-/fast-green-stained sections reveal that, in *Kif3a*-deficient mice (K), articular cartilage (ac) is thin and is partially fused to the articular disc (ad), and subchondral bone (sb) has fewer trabeculae and more marrow space compared with controls (E). Back view of mutant mandible reveals that the mandibular symphysis joint is narrow and accompanied by elongated incisors and a laterally opened mandibular ramus (L). Scale bars: 0.5 cm in G for A, F, G, L; 0.6 mm in H for B, C, D, H, I, L; 250 μ m in E for E, L.

readily seen in superficial/polymorphic cells (Figs. 2E, 2F), verifying that *Col2a1-Cre* had acted specifically in chondrocytes and that *Kif3a* is indeed essential for ciliogenesis.

Zonal Organization Is Altered in *Kif3a*-deficient Condyles

To gain insights into the mechanisms of condylar defects and reduced medio-lateral axis growth, we carried out *in situ* hybridization analyses. By P0, expression of *collagens type II* (*Col II*) and *type X* (*Col X*) in wild-type condyles was characterized by flattened and hypertrophic chondrocytes, respectively. In mutants, the *Col II*-expressing region appeared wider than in controls, an indication of slightly delayed chondrocyte maturation (Appendix Fig. 3). By P15, the wild-type condyles displayed a growth-plate-like structure capped by typical fibrous and polymorphic tissue layers containing chondroprogenitors (Figs. 2I, 3A). Immature *Col II*-expressing chondrocytes not only occupied the upper portion of the structure but were also present along the chondro-perichondrial border (Fig. 2J, arrowheads), and mature *Col X*-positive hypertrophic chondrocytes were arranged in a similar manner (Fig. 2K, arrowheads), suggesting that the seemingly oval shape of each zone reflected the oval contour of the articular surface. In P15 mutants (Fig. 2L), the condyles had become flatter (Fig. 2L, double horizontal arrow), and the short growth-plate-like structure (vertical double arrow) displayed overlapping *Col II/X* expression (Figs. 2M, 2N). Notably, *Col II*- or *Col X*-expressing chondrocytes were barely detectable along the chondro-perichondrial border (Figs. 2M, 2N, arrowheads).

Mutant Polymorphic Layer Is Dysfunctional and Invaded by Periosteum

Because the fibrous/polymorphic cells retain primary cilia in mutants (see Fig. 2F), we asked whether they were phenotypically normal; thus, we monitored markers including fibromodulin (*Fm*), Collagen I (*Col I*), *Col II*, lubricin, and proliferation (*H4C*). In P15 wild-type condyles, *Fm* expression was indeed strong in fibrous/polymorphic cells (Wadhwa *et al.*, 2005) and flattened chondrocyte layer (Figs. 3A, 3B). *Fm* expression expanded in the lateral side of the condyle and continued along the perichondrium (Figs. 3K, 3L, arrowheads) and was followed by *Col I* expression in periosteal cells and osteoblasts in bone collar and endochondral bone (Fig. 3M, arrow). In mutants, the fibrous/polymorphic cell region of *Fm* expression was reduced (Figs. 3F, 3G), and this was particularly evident when the *Fm* hybridization signal (white) was superimposed on the chondrocyte-specific *Col II* signal (red) (Figs. 3C, 3H). In addition, the superficial layer contained uncharacteristic cells with relatively small nuclei and expressed lower levels of lubricin (Figs. 3E, 3J), an extracellular protein important for joint lubrication whose reduced levels could have contributed to partial fusion of the articular disc and condylar surface in the mutants (see Fig. 1L). Equally importantly, mutant condyles displayed ectopic bone collar formation and *Col I*-positive osteoblasts reaching the polymorphic layer (Figs. 3O, 3Q, arrow) and reduced mitotic activity assessed by *H4C* expression in both the central fibrous/polymorphic cell layer ($p < 0.05$) (Figs. 3D, 3I, Appendix Fig. 2) and the lateral tissues ($p < 0.001$) (Figs. 3N, 3R, Appendix Fig. 4).

Figure 2. Primary cilia presence and growth-plate-like zonal organization are abnormal in *kif3a*-deficient mandibular condyles. Frontal sections of P7 control (A-D) and *Kif3a*-deficient (E-H) condyles were processed for safranin O/fast green staining (A, E) and immunodetection of acetylated α -tubulin (B-D, F-H); boxed areas in A and E are shown at higher magnification in B-D and F-H, respectively. Note that primary cilia are still present in mutant superficial/polymorphic layer cells (F), but are barely detectable in flattened (fc) and hypertrophic (hc) chondrocytes (G, H) compared with controls (C, D). Frontal sections from P15 control (I-K) and *Kif3a*-deficient (L-N) condyles were processed for safranin-O/fast green staining (I, L) and *in situ* hybridization (J-K, M-N) with isotope-labeled riboprobes for type II collagen (*Col II*) (J, M) and type X collagen (*Col X*) (K, N). *Kif3a*-deficient condyles exhibit a short growth-plate-like structure (gp) (L, vertical double arrow) and are thinner (L, horizontal double arrow). *Col II* and *Col X* expression (M, N, respectively) largely overlaps in *Kif3a*-deficient mice but not in controls (J, K, respectively). Scale bars: 150 μ m in A for A, E; 20 μ m in B for B, C, F, G; 35 μ m in D for D, H; and 200 μ m in K for I-N.

Ectopic *Ihh* Signaling near the Polymorphic Cell Layer

To clarify the mechanisms for ectopic bone formation, we analyzed *Ihh* expression and signaling. Compared with controls (Figs. 4A, 4B), *Ihh* transcripts were more abundant, characterized the precociously enlarged chondrocytes, and were accompanied by strong *Ptch1* expression in surrounding perichondrial tissues (Figs. 4E, 4F arrowheads), suggesting that these tissues may have shifted from being chondrogenic to being osteogenic. To test this possibility, we monitored the expression of the chondrogenic master regulator *Sox9* and the osteogenic master regulator *Osterix* (*Osx*) (Akiyama *et al.*, 2002; Nakashima *et al.*,

2002). Indeed, while *Sox9* expression was much reduced in mutants compared with controls (Figs. 4C, 4G), *Osx* expression was markedly increased and even reached the apical articular region (Figs. 4D, 4H).

DISCUSSION

Analysis of our data clearly indicates that *Kif3a* and primary cilia are required for development, morphogenesis, and growth of condylar cartilage. Based on previous work from this and other laboratories (Haycraft *et al.*, 2007; Koyama *et al.*, 2007; Song *et al.*, 2007), their roles can be depicted and integrated in

Figure 3. *Kif3a*-deficient condyles display abnormal superficial/polymorphic cell layer function and ectopic osteogenesis reaching the polymorphic layer. Serial frontal sections from P15 control (A-E, K-N) and *Kif3a*-deficient (F-J, O-R) condyles were processed for safranin O/fast green staining (A, F, K, O) and *in situ* hybridization (B-E, G-J, L-N, P-R) with the isotope-labeled riboprobes for fibromodulin (*Fm*) (B, G, L, P), *Col II* (C, H), *H4C* (D, I, N, R), *lubricin* (E, J), and *Col I* (M, Q). The central (A-J) and lateral portions (K-R) of control and *Kif3a*-deficient condyles are magnified to show their zonal organization and chondrocyte maturation zones, and dotted lines indicate the outermost contour of the condyles (B, C). Note that in *Kif3a*-deficient condyles, the *Fm*-expressing polymorphic cell layer (G) is reduced in height compared with that in controls (B), and this is particularly evident after computer-assisted representation of *Fm* expression in white grains and *Col II* expression in red grains (compare H with C). Note also the reduction of *lubricin* expression in both the mutant superficial layer and articular disc (J). Along the lateral aspect of condyles (K-R), *Fm*-expressing polymorphic layer cells gradually merge into perichondrium (K, L, arrowheads), while periosteum and bone collar are characterized by strong *Col I* expression (K, M, arrow). Fast-green-stained bone matrix is detected near the *Kif3a*-deficient condylar head (O, arrow) and is accompanied by an expanded *Col I*-expressing periosteum (Q, arrow) and a narrower *Fm*-expressing polymorphic layer (P, arrowhead). The number of *H4C*-positive chondroprogenitors in the lateral portion of *Kif3a*-deficient polymorphic layer is drastically decreased (R, arrowhead) but is less affected in the central portion of the condyle (I). *ad*, articular disc; *sf*, superficial layer; *pm*, polymorphic layer; *fc*, flattened chondrocyte layer; *hc*, hypertrophic chondrocyte layer. Scale bars: 90 μ m in F for A-J and 120 μ m in O for K-R.

the following working model (Fig. 4I, left schematic). In normal condyles, the polymorphic cell layer is thick, contains abundant *Sox9*-expressing chondroprogenitor cells, and covers the oval-shaped condylar end, but becomes thinner laterally where it merges seamlessly with perichondrium. Distinct condylar chondrocyte zones lie beneath the polymorphic layer, including the hypertrophic zone, where *Ihh* is produced and from which *Ihh* diffuses into the polymorphic layer itself to regulate proliferation and chondrogenesis and into periosteal tissues to induce bone collar formation. Thus, post-natal condyles establish clear, distinct domains—chondrogenesis (polymorphic layer) and osteogenesis (periosteum)—and normal condylar development and growth could require coordinated interplays between chondrocytes and those domains. In *Kif3a*-deficient condyles in which cilia are selectively ablated in chondrocytes, coordination of all these processes is disrupted. The polymorphic layer is thinner and displays low mitotic activity, and chondrocytes undergo precocious maturation and hypertrophy. Because *Ihh* is still produced but does not appear to be under normal topographical regulation, it diffuses broadly and triggers ectopic osteogenesis in the perichondrium and polymorphic layer, turning the chondrogenic field into an osteogenic field. As a consequence, both condylar overall morphology and growth are hampered, likely due to reduced chondroprogenitor cell proliferation and chondrogenesis along the antero-posterior and medio-lateral axes (Fig. 4I, right schematic).

The consequences of conditional *Kif3a* deletion in condyles appear to be milder than those elicited on long bones at post-natal stages (Haycraft et al., 2007; Song et al., 2007). In mutant long bone growth plates, *Ihh* signaling was totally disrupted, but we found that *Ihh* expression in early hypertrophic chondrocytes was maintained, and that *Hh* target cells in the chondroprogenitor layer and perichondrium still responded to *Hh* signaling. One plausible explanation for

these differential effects is the following. In mutant long bones, *Kif3a* and Hh responsiveness was disrupted by *Col2Cre* action in the columnar chondrocytes that are a major contributor to longitudinal growth (St-Jacques *et al.*, 1999; Maeda *et al.*, 2007). In mutant condyles, however, *Kif3a* was not deleted by *Col2Cre* in the chondroprogenitor cell layer that is a major target of Hh signaling (Shibukawa *et al.*, 2007; Ochiai *et al.*, 2010). Though not completely normal, appositional growth due to chondroprogenitor proliferation and chondrogenesis and longitudinal growth could thus continue.

We found that primary cilia in the superficial/polymorphic layers, which functionally resemble articular cartilage in long bones, are occasionally appreciable on the superior, inferior, or lateral side of the cells. Recent detailed analyses on long bone articular cartilage has revealed that the axoneme of primary cilia in superficial and/or radiant zone chondrocytes has a definable orientation and is often directed toward the subchondral bone or articular surface (Farnum and Wilsman., 2011). The difference in ciliar location in condylar superficial/polymorphic cells vs. long bone articular cartilage may be related to the different organization and composition of the matrix in the two tissues: cell-rich vs. cell-sparse; fibrogenic cells vs. permanent chondrocytes; and type I collagen-containing matrix vs. type II collagen-/aggrecan-rich cartilage matrix, respectively (Ochiai *et al.*, 2010). Intriguingly, ciliar organization and orientation could also reflect functional differences. Condylar cartilage is well-known for its adaptability to remodeling in response to external stimuli during development and growth or even after growth (Rabie *et al.*, 2003; Wattanachai *et al.*, 2009). Thus, the variable location of primary cilia in condylar cells may allow for greater mechanical sensitivity and superior adaptability to remodeling. A recent study has indicated that the preferential orientation of primary cilia in the growth plate is lost in growth-plate-associated osteochondromas, suggesting that ciliar topographical location could in fact influence cell function (de Andrea *et al.*, 2010). Variable orientation and positioning of primary cilia in mandibular condyles indicate that a strict and defined ciliary orientation is not needed to transduce the gradient of *Ihh* signaling in mandibular condyles.

The overall contour of the *Kif3a*-deficient condyle is relatively flat compared with the very round contour in controls, and this change becomes detectable by P15. During the short two-week period from birth to P15, we found that control condyles become enriched with a large number of *Col II*-expressing flattened/immature chondrocytes, and the shape of the chondrocyte zones as well as the polymorphic layer is round/oval as well. This implies that the mitotic activity of chondroprogenitors and chondrogenesis are closely associated with underlying chondrocyte zonal organization and function and could establish overall condylar morphology. The two-week post-natal period is also the time when condyles become subjected to significant mechanical load during lactation. Given that mutant flattened chondrocytes are defective in ciliogenesis and undergo early hypertrophy, biomechanical signals transmitted to flattened chondrocytes' cilia would not be fully perceived, and such failure could, in turn, alter chondroprogenitor proliferation, chondrogenesis, and osteogenesis in conjunction with concomitant changes in Hh signaling and function.

Figure 4. Alteration of *Ihh* signaling topography is associated with ectopic bone formation in *Kif3a*-deficient condyles. Serial frontal sections from P15 control (A-D) and *Kif3a*-deficient (E-H) condyles were processed for *in situ* hybridization with isotope-labeled riboprobes for *Indian hedgehog* (*Ihh*) (A, E), *Patched1* (*Ptc1*) (B, F), *Sox9* (C, G), and *Osterix* (*Osx*) (D, H). In control condyles: *Ihh* transcripts are present in early hypertrophic chondrocytes (A); *Ptc1* expression is evident in Hh-target cells in superficial/polymorphic and perichondrium (B, double arrowhead); *Sox9* expression characterizes chondroprogenitors in the polymorphic layer and newly differentiated chondrocytes (C); and strong *Osx* expression is evident in periosteum and overlaps *Ptc1* expression (B, D, double arrowheads). In mutant condyles, *Ihh* is strongly expressed in the upper portion of condylar cartilage and is accompanied by strong *Ptc1* expression in surrounding tissues (F). *Sox9*-expressing cells in the polymorphic layer are significantly reduced, while *Osx*-expressing osteogenic cells become abundant and ectopic and reach the apical condylar *Ptc1*-expressing region (F, H, double arrowheads). (I) Schematics depict our current working models for post-natal condylar organization and function in wild-type (left) vs. *Kif3a*-deficient (right) mice (see text for details). Scale bar: 250 μ m in A for A-H.

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