

Selective Role of Discoidin Domain Receptor 2 in Murine Temporomandibular Joint Development and Aging

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Abstract

Temporomandibular joint (TMJ) disorders are often associated with development of osteoarthritis-like changes in the mandibular condyle. Discoidin domain receptor 2 (DDR2), a collagen receptor preferentially activated by type I and III collagen found in the TMJ and other fibrocartilages, has been associated with TMJ degeneration, but its role in normal joint development has not been previously examined. Using *Ddr2* LacZ-tagged mice and immunohistochemistry, we found that DDR2 is preferentially expressed and activated in the articular zone of TMJs but not knee joints. To assess the requirement for *Ddr2* in TMJ development, studies were undertaken to compare wild-type and *smallie* (*slic*) mice, which contain a spontaneous deletion in *Ddr2* to produce an effective null allele. Analysis of TMJs from newborn *Ddr2^{slic/slic}* mice revealed a developmental delay in condyle mineralization, as measured by micro-computed tomography and histologic analysis. In marked contrast, knee joints of *Ddr2^{slic/slic}* mice were normal. Analysis of older *Ddr2^{slic/slic}* mice (3 and 10 mo) revealed that the early developmental delay led to a dramatic and progressive loss of TMJ articular integrity and osteoarthritis-like changes. Mutant condyles had a rough and flattened bone surface, accompanied by a dramatic loss of bone mineral density. Mankin scores showed significantly greater degenerative changes in the TMJs of 3- and 10-mo-old *Ddr2^{slic/slic}* mice as compared with wild-type controls. No DDR2-dependent degenerative changes were seen in knees. Analysis of primary cultures of TMJ articular chondrocytes from wild-type and *Ddr2^{slic/slic}* mice showed defects in chondrocyte maturation and mineralization in the absence of *Ddr2*. These studies demonstrate that DDR2 is necessary for normal TMJ condyle development and homeostasis and that these DDR2 functions are restricted to TMJ fibrocartilage and not seen in the hyaline cartilage of the knee.

Keywords: temporomandibular disorders (TMD), growth/development, joint disease, craniofacial biology/genetics, cartilage, cell-matrix interactions

Introduction

Temporomandibular joint (TMJ) disorders affect 10% to 40% of the US population. A large proportion of affected individuals develop osteoarthritis (OA)-like degenerative changes that are characterized by cartilage degradation, osteophyte formation, and joint mineralization (Carlsson 1999; Liu and Steinkeler 2013). Although TMJ-OA shares a similar pathology with OA in other joints, it also has unique characteristics including early onset and preferential occurrence in women. This is to be contrasted with OA in hyaline cartilage joints such as the knee, which increases with age and exhibits no sexual preference (Zhang and Jordan 2010). The basis for these differences in OA etiology remains unknown.

We believe that an understanding of interactions between TMJ articular fibrochondrocytes and their extracellular matrix may provide critical cues for understanding unique aspects of TMJ development and pathogenesis. Unlike hyaline cartilage extracellular matrix, which principally contains type II collagen, TMJ fibrocartilage also contains appreciable amounts of type I and III collagen (Wiberg and Wanman 1998; Arden and Nevitt 2006). Discoidin domain receptor 2 (DDR2) is an important mediator of cell-collagen interactions that may be important for understanding interactions between TMJ fibrochondrocytes and collagen. The discoidin domain receptor tyrosine kinases DDR1 and DDR2 each have a unique tissue

distribution and sensitivity to activation by specific collagens. DDR1 is expressed in epithelium and weakly activated by collagens I through IV. In contrast, DDR2 is predominantly expressed by mesenchymal cells and is strongly activated by type I and III collagen but is less responsive to collagens II, IV, and V (Vogel et al. 1997; Benjamin and Ralphs 2004). Because DDR2 is preferentially activated by collagens found primarily

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This article was initially published OnlineFirst with A. Binrayes's second affiliation with King Saud University omitted. This has now been added and the following affiliations have been renumbered accordingly. This has been corrected online and in print.

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in TMJ fibrocartilage, we hypothesized that it has a unique and specific role in TMJ development and contributes to the pathogenesis of TMJ degeneration.

Here we use a genetic approach to address this concept by studying the distinct expression pattern of DDR2 in the TMJ, evaluating the early effects of DDR2 deficiency on TMJ versus knee joint development as well as the long-term consequences of deficiency on TMJ cartilage and bone. As shown, DDR2 has a unique role in TMJ development and aging.

Materials and Methods

Animals

Ddr2 LacZ-tagged mice were used to determine the distribution of *Ddr2* expression. Mice were developed from embryonic stem cell clone *Ddr2*^{tm1a(EUCOMM)Wtsi} (EPD0607__B01) obtained from the European Mutant Mouse Repository. This clone contains a “knockout first” allele in the endogenous mouse *Ddr2* locus containing a bacterial *LacZ* cassette and neomycin resistance gene 5' to exon 2 of *Ddr2* with appropriately placed loxP and FLP sites (see Fig. 1A for map). Crossing mice containing this allele with mice containing a Cre expressed in the germline, such as *Sox2-Cre*, leads to excision of the neo cassette and exon 2 to generate a mouse expressing *LacZ* under the control of the endogenous *Ddr2* gene. Embryonic stem cell transplantation was performed by the University of Michigan Transgenic Model Core. *Ddr2* LacZ-tagged mice were developed by crossing knockout-first allele mice with *Sox2-Cre* mice (Hayashi et al. 2002; Fig. 1A). Two-month-old male heterozygous *Ddr2* LacZ-tagged mice (*Ddr2*^{+/LacZ}) maintained in a C57BL/6 background were used for LacZ analysis. All studies examining effects of *Ddr2* deficiency used *Smallie* mice (*Ddr2*^{slie/slie} mice), which contain a spontaneous 150-kb deletion in *Ddr2* that removes exons 2 through 17 to produce an effective null allele (Kano et al. 2008). Mice were obtained from the Jackson Laboratory, *BKS(HRS)-Ddr2*^{slie/}*JngJ*, and bred into a C57BL6 background for at least 10 generations (Ge et al. 2016). Mice were genotyped with a quantitative real-time polymerase chain reaction (qPCR) protocol provided by Jackson Labs. Whole skull and knee joints were dissected and analyzed from newborn, 3-, and 10-mo-old wild-type (WT) and *Ddr2*^{slie/slie} mice ($n = 6$ to 8).

TMJ and Knee Joint Chondrocyte Isolation for RNA Analysis

Articular chondrocytes were isolated from 12-wk-old WT mice with an established method (Gosset et al. 2008). Briefly, mandibular condyles and tibial plateaus were exposed by removing capsules and disc/meniscus. The articular cartilage was cut from mandible condyle and tibia along the mandible/tibial neck. Articular chondrocytes were then isolated by collagenase A digestion and total RNA extracted with TRIzol reagent for mRNA analysis by reverse transcription quantitative real-time PCR (RT-qPCR).

Micro-computed tomography Analysis of Bone

Whole skull and knee joints were scanned by micro-computed tomography (μ CT) with a Scanco Model 100 (Scanco Medical). Scan settings were as follows: voxel size of 12 μ m, 70 kVp, 114 μ A, 0.5-mm aluminum filter, and integration time of 500 ms. All scans were analyzed with fixed thresholds (180 for bone volume). For quantification of subarticular bone volume of TMJ condyles and tibia heads, the mineralized tissue volume of each section was measured between the process from anterior to posterior and the line along the mandible/tibia neck. Total bone volume was determined by adding all the sections between the exterior and interior of each joint.

Tissue Preparation and Histopathologic Analysis

For whole mounts, tissue was fixed in 2% paraformaldehyde, 0.25% glutaraldehyde, and 0.01% NP40 in phosphate-buffered saline, while for tissue sections, samples were first decalcified with 10% ethylenediaminetetraacetic acid for 1 wk and then embedded in OTC and frozen. Fixed tissue and frozen sections were incubated with 1 mg/mL of X-gal overnight. Whole mount alizarin red and alcian blue staining and tissue clarification were conducted as previously described (Ge et al. 2007). For immunohistochemistry, tissue was fixed in 4% formalin and embedded in paraffin. Sections were incubated with total DDR2 antibody (ab5520; Abcam) or phospho-DDR2 (Y740) antibody (MAB25382; R&D). For safranin O staining, paraffin-embedded sections were stained with 0.001% fast green and 0.1% safranin O. A modified Mankin scoring system was used to evaluate pathologic changes in TMJ and knee articular cartilage (Xu et al. 2009; Xu et al. 2011).

Cell Cultures and In Vitro Differentiation

Primary articular chondrocytes were harvested from TMJ condyles dissected from 3-mo-old WT and *Ddr2*^{slie/slie} mice as described previously (Park et al. 2015). Primary cells were stimulated to undergo chondrocyte differentiation by culturing for 15 d in α -MEM/10% fetal bovine serum containing 50 μ g/mL of ascorbic acid, 10mM β -glycerophosphate, and 20 μ g/mL of TGF- β 1. Visualization of chondrocyte hypertrophy was performed by alcian blue staining and immunostaining with collagen X antibody (ab58632; Abcam) at day 5. Mineralization was assessed by alizarin red S staining (Ge et al. 2016). Cell images were taken with an inverted phase contrast microscope (Nikon D300). To measure mRNA expression, total RNA was isolated at the times indicated, and mRNA levels were measured by RT-qPCR.

Statistical Analysis

All statistical analyses were performed with SPSS 16.0 (IBM). Values are presented as mean \pm SD. For in vivo studies, at least 6 animals were used per group. For cell culture studies, triplicate independent samples were used. Statistical significance

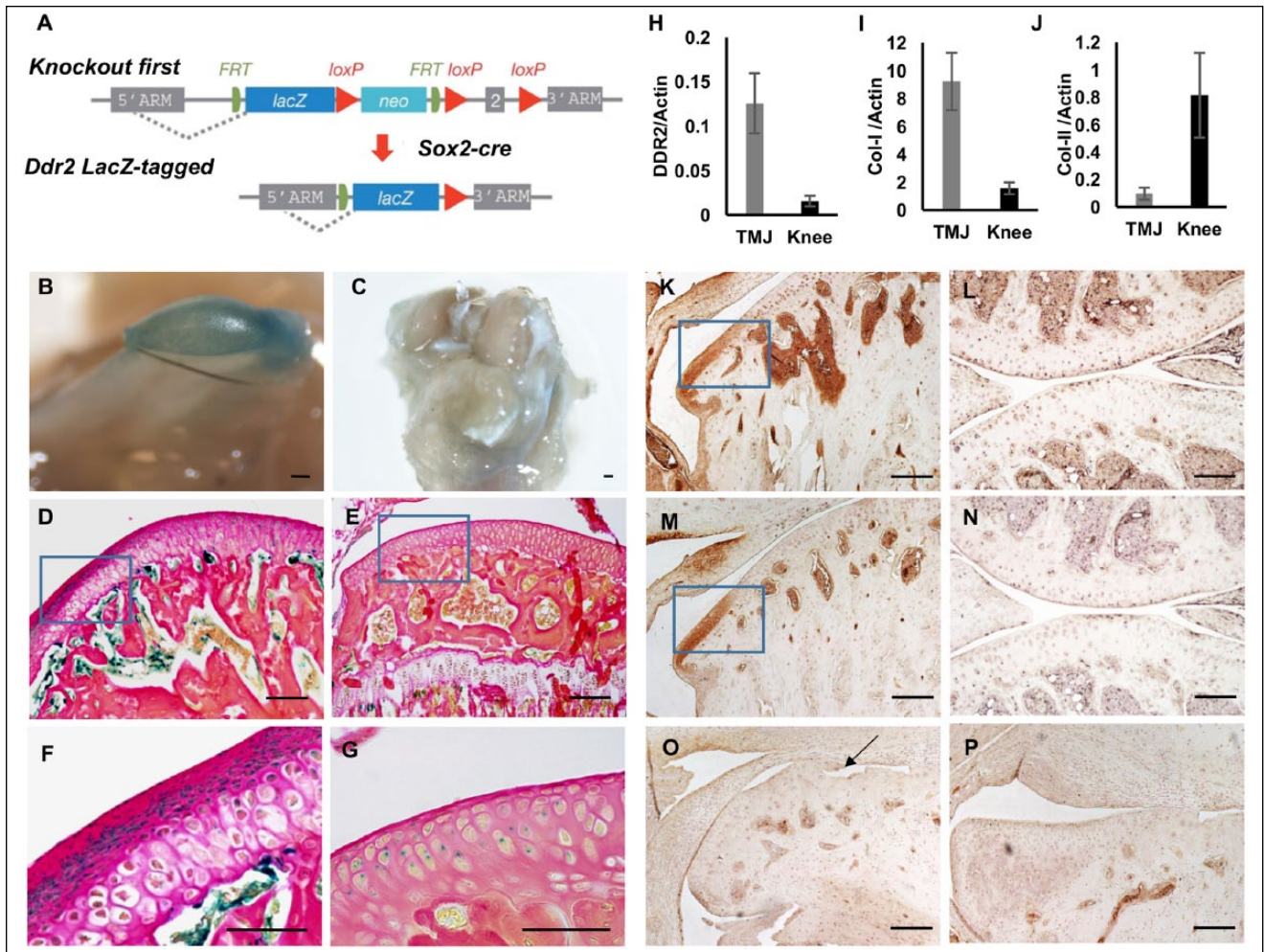


Figure 1. Discoidin domain receptor 2 (DDR2) is preferentially expressed and activated in temporomandibular joint (TMJ) articular fibrocartilage. (A) Strategy for developing *Ddr2* LacZ-tagged allele mice. Mice with recombinant allele knocked into the *Ddr2* locus (“knockout first” allele) were developed as described in the methods and crossed with global Cre (*Sox2-Cre*) mice to generate a germline *Ddr2* LacZ-tagged allele (*Ddr2*^{+LacZ}). (B–G) LacZ localization in mandibular condyle (B, D, F) and knee joint (C, E, G) from 2-mo-old *Ddr2*^{+LacZ} mice. Whole mount LacZ staining (B, C). Low- (D, E) and high-magnification (F, G) histologic sections; high-magnification images are of boxed areas in D, E. (H–J) Reverse transcription quantitative real-time polymerase chain reaction detection of *Ddr2* (H), *Col1a1* (I), and *Col2a1* (J) mRNA in TMJ and knee articular cells from 2-mo-old wild-type mice (*n* = 6). Values were normalized to β -actin mRNA. (K–P) Immunohistochemistry: TMJ (K, M, O, P) and knee joint (L, N). Antibodies: anti-total DDR2 (K, L, O) and anti-phospho-DDR2 (Y740; M, N, P). (O, P) To determine background staining, TMJs from *Ddr2*^{slie/slle} mice were stained with total (O) and phospho-DDR2 (P) antibodies. Scale bars: 0.2 mm in panel B; 0.5 mm for panel C; 40 μ m for panels D, E, K–P; 20 μ m for panels F, G. Arrow (O) indicates defects in condylar morphology of *Ddr2*^{slie/slle} mice.

was assessed with 1-way analysis of variance. $P < 0.05$ is considered a significant difference between groups.

Results

DDR2 Is Preferentially Expressed and Activated in the Articular Zone of the TMJ

DDR2 has been localized to mesenchymal cells including osteoblasts, chondrocytes, muscle, ligaments, and gonadal tissue (Leitinger 2014). However, its detailed expression pattern in fibrocartilage joints such as the TMJ and in hyaline cartilage joints including the knee has not been previously examined. To address this issue, a *Ddr2*-LacZ mouse line was generated from knockout-first *Ddr2*-targeted embryonic stem cell clones as

described in the methods. As shown by whole mount LacZ staining of heterozygous *Ddr2* LacZ-tagged mice, *Ddr2* was strongly expressed in the TMJ condyle with little or no staining in the articular surface of the knee joint (Fig. 1B, C). LacZ staining of histologic sections showed *Ddr2* expression in the TMJ articular surface, where multiple layers of cells exist (Fig. 1D, F). In contrast, the knee joint articular surface contains only a single cell layer that has little or no LacZ staining (Fig. 1E, G). Weak LacZ staining was detected in the subarticular zones in the TMJ and knee joint (Fig. 1F, G). The localized expression of DDR2 protein in the TMJ condyle articular surface was confirmed by immunohistochemistry with anti-total DDR2 antibody (boxed area, Fig. 1K). Staining was observed in disc and mandibular (glenoid) fossa regions, although this was not investigated in detail. Consistent with the LacZ

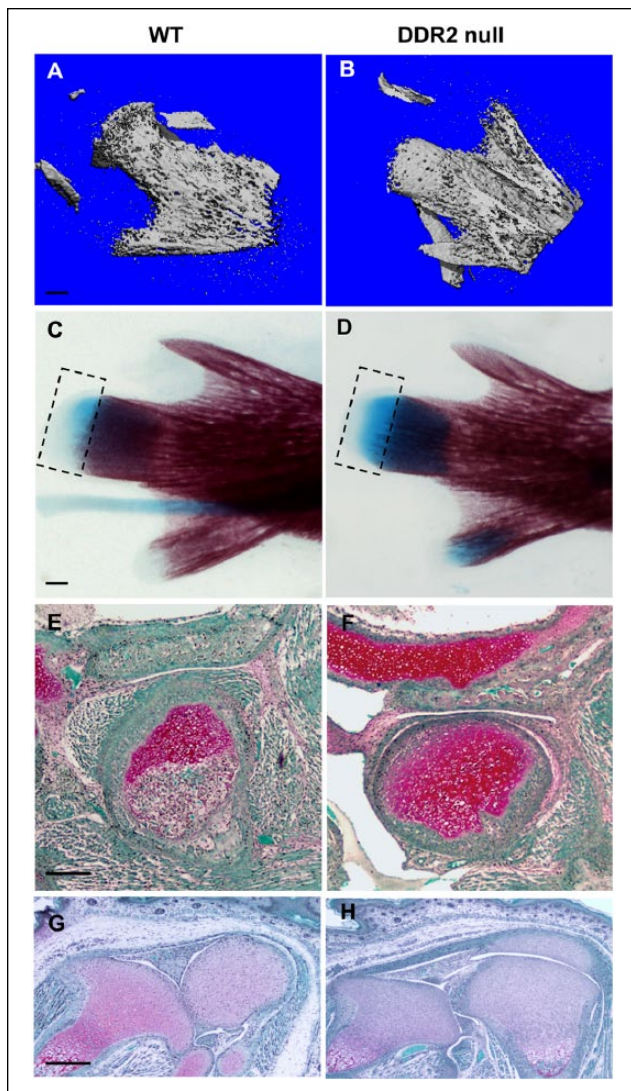


Figure 2. *Ddr2*-deficient mice exhibit tissue-selective defects in temporomandibular joint (TMJ) development. TMJ condyle structure was compared in newborn wild-type (WT) and *Ddr2*^{slie/slie} mice with 3-dimensional reconstructed micro-computed tomography images (A, B), alizarin red and alcian blue whole mount staining (C, D), and safranin O staining of TMJ sections (E, F). Safranin O staining of knee joint histologic sections is also shown (G, H). Scale bars: 0.2 mm in panel A (A, B); 0.5 mm in panel C (C, D); 40 μ m in panel E (E, F); 100 μ m in panel G (G, H). Boxed area in panels C and D indicates alcian blue-stained region.

distribution, little staining was seen in sections of the knee joint articular surface (Fig. 1I). Separate studies isolated chondrocytes from condyles and knee joints (tibial surface), and *Ddr2* mRNA levels were measured by RT-qPCR. Consistent with LacZ staining and immunohistochemical analysis, *Ddr2* mRNA was enriched 10-fold in chondrocytes from the TMJ relative to the knee (Fig. 1H). When compared with the knee joint, TMJ samples were enriched in *Coll1a1* and depleted in *Col2a1* mRNA, as would be expected for this fibrocartilage (Fig. 1I, J).

DDR2 is preferentially activated and autophosphorylated at Y740 by type I and III collagen found in TMJ fibrocartilage

but is less responsive to collagens II, IV, and V (Ikeda et al. 2002; Yang et al. 2005). To determine if TMJ-associated DDR2 is in a phosphorylated, activated state, immunohistochemistry was conducted with a phospho-Y740 DDR2 antibody. As shown in Figure 1M, strong phospho-DDR2 staining was seen in the TMJ articular zone and disc. In contrast, phosphorylated DDR2 was undetectable in the articular zone of the knee joint (Fig. 1N). The specificity of total and P-DDR2 antibody staining was confirmed by their inability to stain TMJ sections from *Ddr2*^{slie/slie} mice (Fig. 1O, P).

DDR2 Deficiency in *Ddr2*^{slie/slie} Mice Causes a Selective Delay in TMJ Development

To determine the consequences of global *Ddr2* deficiency in TMJ development, we examined WT and *Ddr2*^{slie/slie} mice. These animals contain a spontaneous 150-kb deletion in the *Ddr2* gene to produce an effective null allele (Kano et al. 2008). As shown in our previous study, *Ddr2*^{slie/slie} mice have major defects in craniofacial, trabecular, and, to a lesser extent, cortical bone formation (Ge et al. 2016). The preferential expression of *Ddr2* in the TMJ versus knee joint suggests that it may also have selective functions in TMJ development. In the study shown in Figure 2, we compared TMJ and knee joints from newborn *Ddr2*^{slie/slie} mice with WT littermates. Mineralization of the mandibular condyle as measured by μ CT was delayed in *Ddr2*^{slie/slie} mice (Fig. 2A, B). Whole mounts showed a larger volume of unmineralized cartilage (alcian blue stain) in mutant condyles (Fig. 2C, D, boxed area). Consistent with these findings, histologic analysis showed the TMJ condyle to contain safranin O-staining chondrocytes (red) in newborn *Ddr2*^{slie/slie} mice, while cartilage in condyles from WT mice had largely been replaced by mineral (Fig. 2E, F). Mineralization of glenoid fossa articular surfaces within the temporal bone was delayed in *Ddr2*^{slie/slie} mice (Fig. 2E, F). In contrast, knee joints from WT and *Ddr2*^{slie/slie} mice were indistinguishable at this time point (Fig. 2G, H).

Adult *Ddr2*^{slie/slie} Mice Develop Spontaneous and Selective TMJ Degeneration

To determine whether DDR2 deficiency leads to pathologic changes in adult mice, we next characterized TMJ and knee joints from 3- and 10-mo-old mice with μ CT and histopathologic analysis. Clear changes in TMJ structure were evident in 3-mo-old *Ddr2*^{slie/slie} mice when compared with WT littermates. Two-dimensional μ CT analysis showed a dramatic loss of subchondral bone (Fig. 3A, B). These phenotypic bone changes were even more extensive in 10-mo-old *Ddr2*^{slie/slie} mice, where loss of articular integrity and subchondral bone was observed (Fig. 3C, D). Three-dimensional μ CT reconstruction of whole mandibular condyles showed severe bone loss on the condyle surface from 3-mo-old *Ddr2*^{slie/slie} mice (Fig. 3E, F, I, J). By 10 mo of age, the condyle surface became flattened in *Ddr2*^{slie/slie} mice due to loss of subchondral bone (Fig. 3G, H, K, L). Histopathologic analysis further confirmed

loss of tissue integrity starting in 3-mo-old *Ddr2^{slie/slie}* mice (arrows in Figs. 1O, 3N) that was progressively more severe in 10-mo-old animals. The pathologic changes included a decrease in safranin O–stained proteoglycan (Fig. 3N) that expanded to include >50% of the TMJ condyle surface in 10-mo-old *Ddr2^{slie/slie}* mice (Fig. 3P). Subchondral bone loss is considered to be an early indicator of OA formation (Li et al. 2013). TMJ subchondral bone volume fraction, as measured by μ CT, decreased approximately 50% in 3-mo-old *Ddr2^{slie/slie}* mice and 70% in 10-mo-old animals (Fig. 3Q). The OA severity, quantified with a modified Mankin OA scoring system (Xu et al. 2009), showed small age-dependent degenerative changes in TMJs from WT mice and dramatically higher OA scores in 3- and 10-mo-old *Ddr2^{slie/slie}* animals (Fig. 3R).

In contrast to the profound changes in TMJ cartilage and bone in *Ddr2*-null mice, DDR2 deficiency had no obvious effect on knee joints. μ CT analysis detected slight subchondral bone loss in WT and *Ddr2^{slie/slie}* mice when 3- and 10-mo-old mice were compared (Fig. 4A–H). Although bone volume fraction was reduced by approximately 10% in 3-mo-old *Ddr2*-null mice (Fig. 4M), this was not seen at 10 mo and is in marked contrast to the 50% loss seen in TMJs from *Ddr2*-deficient mice. Additionally, the integrity of the articular surface remained intact, and no defects in proteoglycan staining were apparent (Fig. 4I–L). Histopathologic analysis showed an age-related increase in OA score in knee joints of WT and *Ddr2^{slie/slie}* mice, but the severity of joint degeneration on *Ddr2^{slie/slie}* mice was similar to that of WT animals (Fig. 4N).

DDR2 Deficiency Inhibits Differentiation and Hypertrophy of Primary TMJ Fibrochondrocytes

DDR2 has broad effects on cell proliferation, migration, survival, differentiation, and tissue remodeling (Leitinger 2014). In calvarial and marrow stromal cell cultures, DDR2 deficiency severely reduced differentiation to osteoblasts (Ge et al. 2016), but its possible role in fibrochondrocyte differentiation has not been previously examined. To address this issue, fibrochondrocytes were isolated from WT and DDR2-deficient condyles and grown in differentiation media for 2 weeks. Defects in chondrocyte differentiation/maturation were apparent on visual inspection of histochemically stained cultures (Fig. 5A–C).

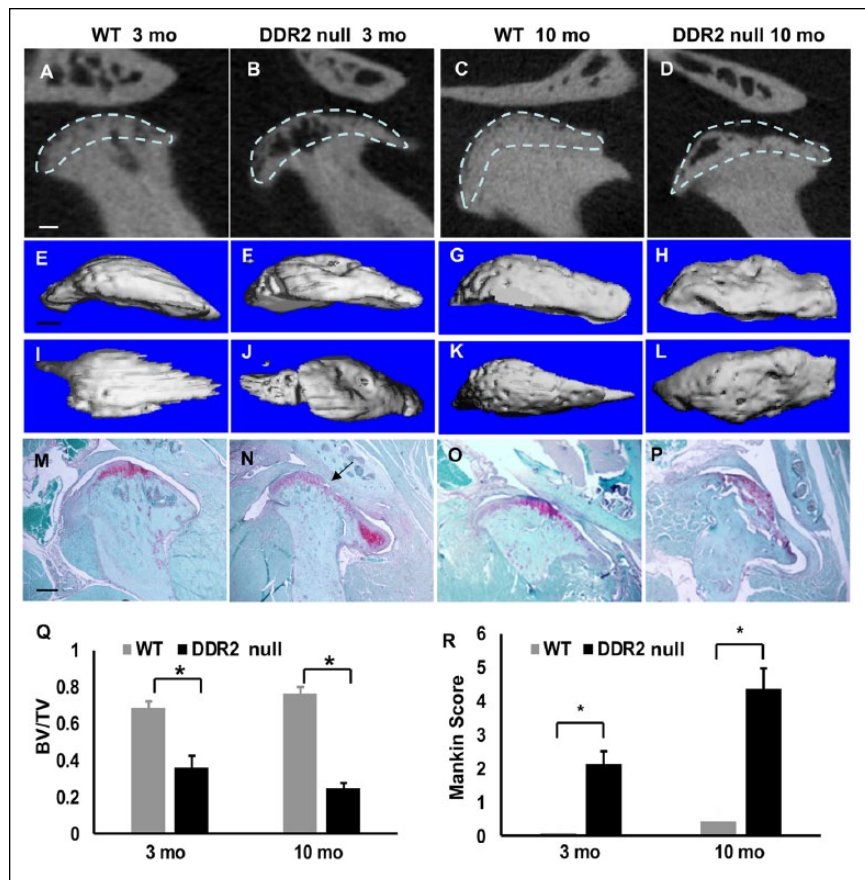


Figure 3. Spontaneous temporomandibular joint (TMJ) degeneration in 3- and 10-mo-old *Ddr2^{slie/slie}* mice. (A–D) Two-dimensional micro-computed tomography (μ CT) reconstruction images of TMJs from 3- and 10-mo-old wild-type (WT) and *Ddr2^{slie/slie}* mice. (E–H) Three-dimensional μ CT lateral views of mandibular condyles. (I–L) Three-dimensional superior views of mandibular condyles. (M–P) Safranin O staining of TMJ sections. Arrow in panel N indicates early degeneration in 3-mo-old DDR2-null section. (Q) μ CT quantification of subchondral bone volume fraction at mandibular condyle surfaces (area of analysis indicated by dashed lines; see Materials and Methods). For panel Q, values are presented as ratio of bone volume / tissue volume; for panel R, as histopathologic score. (R) Histopathologic scoring of TMJ osteoarthritis based on modified Mankin score. Scale bars: 0.25 mm in panel A (A–D); 0.5 mm in panel E (E–L); 40 μ m in panel M (M–P). * $P < 0.05$, $n = 8$.

These findings show that enlarged alcian blue–negative cells having the appearance of hypertrophic chondrocytes were greatly reduced in DDR2-deficient cultures (Fig. 5A). In addition, immunostaining for type X collagen–positive cells and mineralization, additional properties of hypertrophic chondrocytes were reduced (Fig. 5B, C). Consistent with these findings, while chondrocyte markers such as *Col2a1* and *Comp*, which are expressed during early chondrocyte differentiation stages, were not affected by loss of DDR2 (Fig. 5D, E), markers of chondrocyte hypertrophy and maturation, including *Col10a1*, *Mmp13*, *Runx2*, and *Bglap2*, were all significantly decreased in *Ddr2*-deficient cells (Fig. 5F–I).

Discussion

Although DDR2 has been studied as a possible mediator of OA induction, its role in joint development and maturation has not been previously explored. Here we show that DDR2 is preferentially

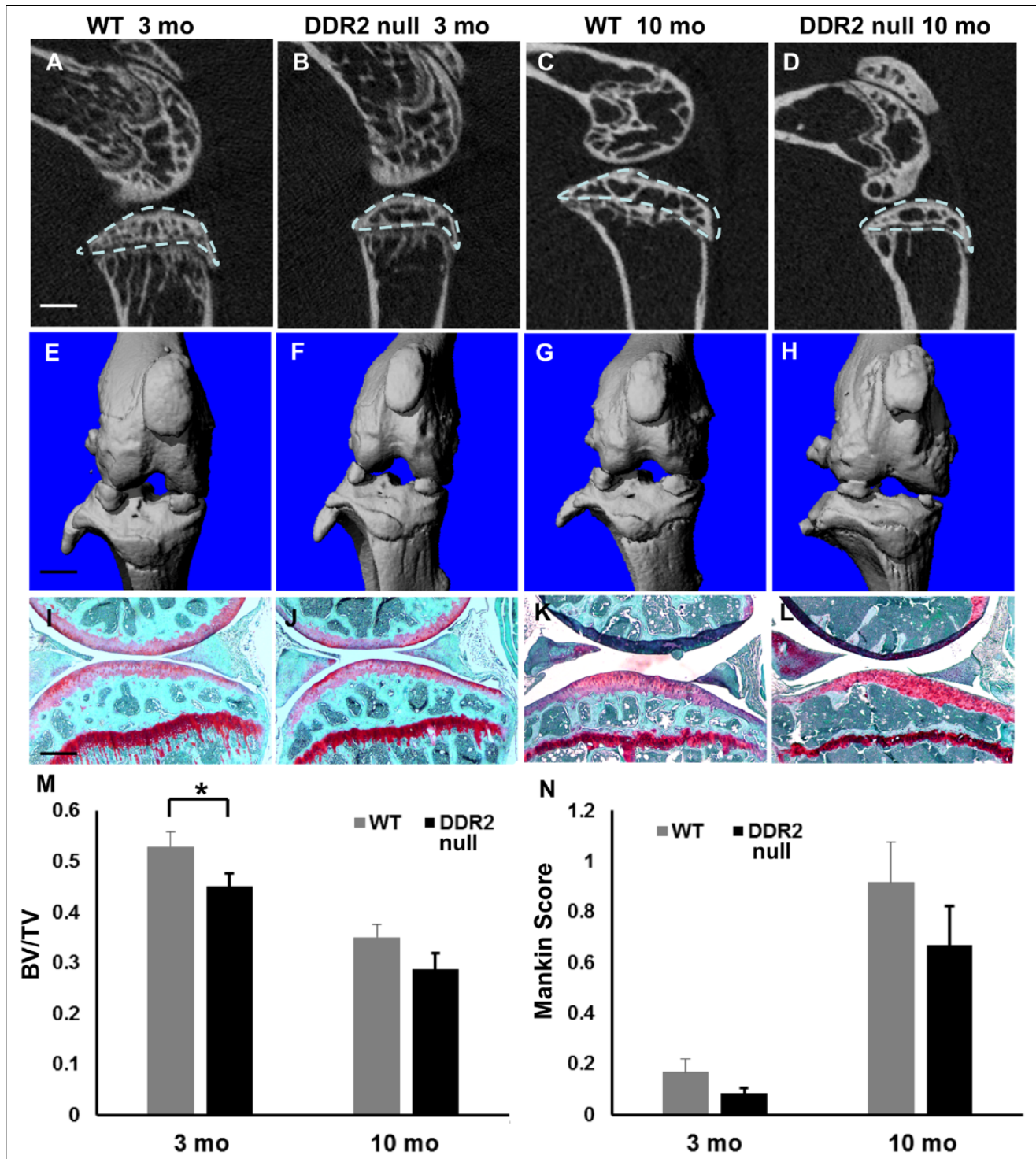


Figure 4. *Ddr2* deficiency does not affect knee joints. (A–D) Micro-computed tomography (μ CT) 2-dimensional reconstruction images of knee joints from 3- and 10-mo-old wild-type (WT) and *Ddr2*^{slie/slue} mice. (E–H) Three-dimensional μ CT images of knee joints. (I–L) Safranin O staining of knee joint sections. (M) μ CT analysis of tibia subchondral bone volume fraction (area of analysis indicated by dashed line in panel A). (N) Histopathologic scoring of knee joint osteoarthritis with modified Mankin score. Scale bars: 0.25 mm in panel A (A–D); 0.25 mm in panel E (E–H); 40 μ m in panel I (I–L). * $P < 0.05$, $n = 6$.

expressed in TMJ fibrocartilage versus hyaline cartilage of the knee. Furthermore, TMJ development is delayed in newborn *Ddr2*-deficient *Ddr2*^{slie/slue} mice, and this developmental defect

leads to spontaneous and progressive TMJ degeneration as animals age. In contrast, DDR2 deficiency does not affect knee joint development or degeneration. This selectivity may be

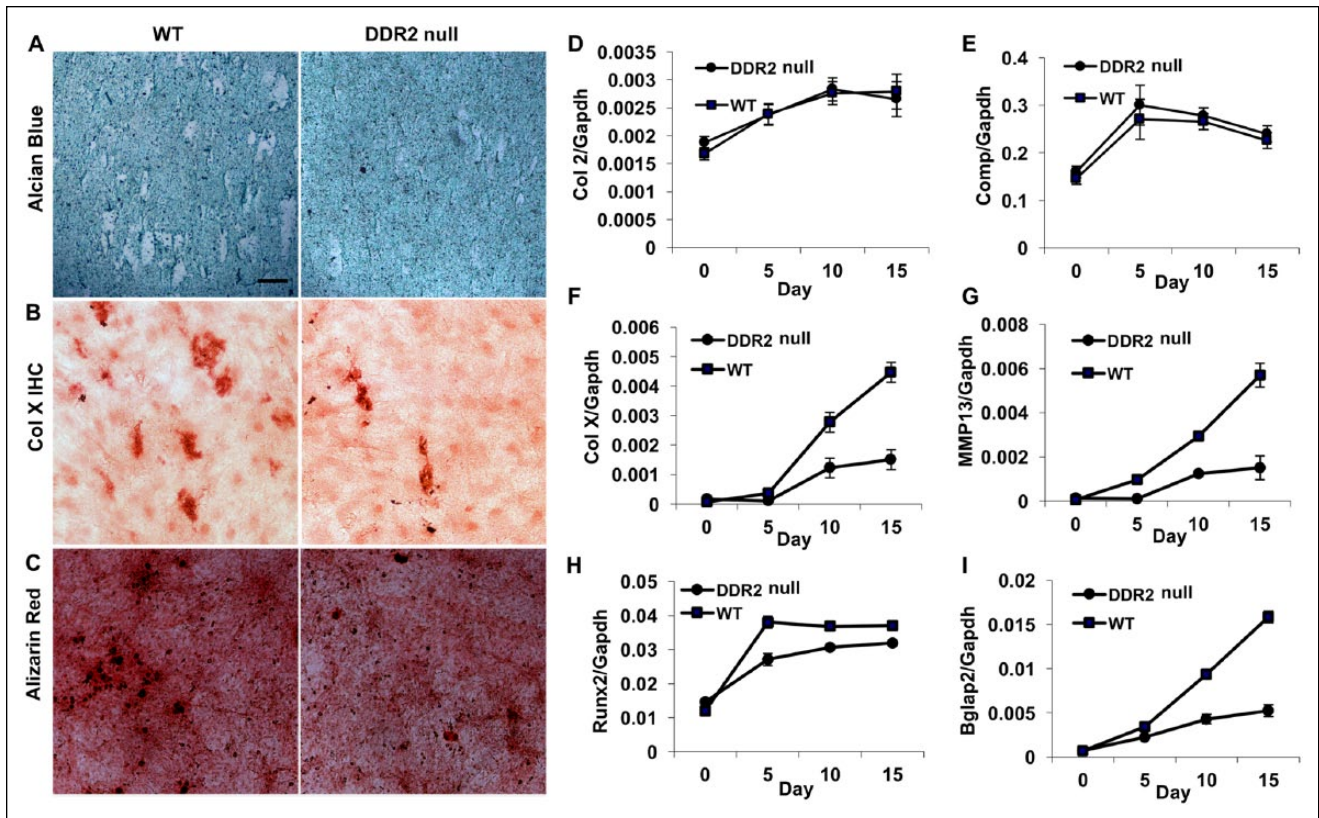


Figure 5. Decreased in vitro differentiation and maturation of primary temporomandibular joint chondrocyte cultures from *Ddr2^{slie/slie}* mice. Primary temporomandibular joint condyle chondrocytes were isolated from 3-mo-old wild-type (WT) and homozygous *Ddr2^{slie/slie}* mice (DDR2) and grown in chondrogenesis medium for up to 15 d. Five-day cultures were used for alcian blue staining (A) and immunohistochemistry for collagen X (B). Fifteen-day cultures were stained for mineral with alizarin red (C). Cultures from day 0 to day 15 were used for reverse transcription quantitative real-time polymerase chain reaction detection of temporal changes in chondrocyte gene expression: (D) *Col2a1* (Col 2), (E) cartilage oligomeric matrix protein (Comp), (F) *Col10a1* (Col X), (G) matrix metalloproteinase 13 (MMP13), (H) runt-related transcription factor 2 (Runx2), and (I) bone gamma carboxylglutamate protein 2 (Bglap 2). Values were normalized to Gapdh mRNA. Scale bar: 40 μ m for panels A–C.

explained by the preferential expression of DDR2 in TMJ versus hyaline joints and specific activation of DDR2 by the type I collagen-enriched extracellular matrix of TMJ fibrocartilage.

DDR2 levels have not been compared between the TMJ and hyaline joints such as the knee. In earlier work, DDR2 was not detectable by immunohistochemistry in healthy knees and was seen only after induction of OA by surgical destabilization (Xu et al. 2010). In our study, levels of DDR2 in knee articular cartilage were very low, whether detected by LacZ staining of *Ddr2^{+LacZ}* mice, immunohistochemistry, or quantitation of *Ddr2* mRNA. In contrast, DDR2 was highly enriched in TMJ condyle fibrocartilage regardless of the detection method used (Fig. 1). The abundance of DDR2 in the TMJ may make it particularly dependent on this collagen receptor for normal development and homeostasis.

Immunohistochemical analysis localized DDR2 to certain cells of the TMJ disc and regions of the glenoid fossa (Fig. 1). This was also seen in LacZ-stained samples (not shown). Furthermore, in addition to delaying condyle mineralization, loss of DDR2 delayed mineralization of the glenoid fossa in newborn mice (Fig. 2). The relationship between these early developmental changes and the subsequent TMJ degeneration shown in Figure 3 is not currently understood. Abnormal

articulation between the condyle and glenoid fossa is clearly apparent even in newborn animals and becomes more severe as animals age (Figs. 2, 3). Furthermore, DDR2 deficiency leads to abnormalities in skull shape with a resulting decrease in the distance between the external occipital protuberance and incisors (Dullin et al. 2007; Kano et al. 2008). These abnormalities, combined with delays in cartilage maturation and mineralization in the condyle and glenoid fossa, could clearly alter the mechanical environment of the TMJ, which is critical for normal maturation and may be related to subsequent joint degeneration (Kuroda et al. 2009; Ding et al. 2010). To determine the relative contribution of DDR2 deficiency-related developmental defects versus possible postnatal functions of DDR2 and to identify critical DDR2-positive cell populations, it will be necessary to utilize tissue-specific inducible *Ddr2* knockout models that are currently under development in the project laboratory.

In a recent study, knockout of the other mammalian discoidin receptor, DDR1, was shown to induce TMJ defects in the absence of knee joint pathology (Schminke et al. 2014). Thus, deficiencies of mammalian discoidin receptors represent the only known mouse genetic models that selectively affect the TMJ. However, *Ddr1*- and *Ddr2*-deficient animals

each exhibit distinct pathologies. In contrast to the early effects of *Ddr2* deficiency, in the *Ddr1* knockout, no TMJ developmental defects were reported. Instead, early-onset TMJ OA was seen beginning at 9 wk of age. The basis for the different phenotypes of DDR1- and DDR2-deficient mice is not known, although, interestingly, loss of DDR1 was accompanied by a compensatory upregulation of TMJ-associated DDR2. We and others previously showed that DDR2 functions to increase bone formation and cartilage hypertrophy (Zhang et al. 2011; Ge et al. 2016). This requirement for DDR2 for cartilage hypertrophy is seen in the results presented in Figure 5, where condyle fibrochondrocytes from *Ddr2^{slie/sl1e}* mice were shown to have a diminished capacity to undergo hypertrophic maturation. It is therefore possible that DDR2 upregulation in *Ddr1*-deficient mice may explain subsequent abnormal TMJ mineralization and OA. Consistent with this concept, surgical destabilization of TMJ or knee joints leads to upregulation of DDR2 and OA induction (Xu et al. 2007), a response that is reduced in mice haploinsufficient for *Ddr2* (Xu et al. 2010).

Taken together, these results are consistent with a model where DDR2 is required for chondrocytic maturation and subchondral bone formation and associated TMJ development. The selective role of DDR2 in TMJ versus knee joint development may be explained by the restricted expression of this collagen receptor in the TMJ articular zone and characteristic responsiveness of DDR2 to the type I and III fibrocartilage collagens present in the TMJ. In contrast, the hyaline cartilage of the knee normally expresses only low levels of DDR2 and is devoid of type I and III collagen necessary for its activation. For this reason, DDR2 may not be necessary for knee joint development. Only after the trauma of surgical destabilization does DDR2 become active in the knee joint, where it can then mediate pathologic mineralization and OA.

Author Contributions

C. Ge, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; F. Mohamed, A. Bin-rayes, contributed to conception, data acquisition, and analysis, critically revised the manuscript; S. Kapila, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript; R.T. Franceschi, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This work was supported by National Institute of Dental and Craniofacial Research Career Development Award K12 DE023574 (C.G.; principal investigator, S.K.) and grant DE11723 (R.T.F.), as well as a scholarship from the Ministry of Higher Education and Scientific Research, Libyan Transitional Government (F.M.), and a scholarship from King Saud University (A.B.). The authors thank Dr. Yuji Mishina (University of Michigan School of Dentistry) for the generous gift of Sox2-Cre mice. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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