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EDITORIAL

What Makes the Permanent Articular Cartilage Permanent?

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Articular cartilage functions by providing a specialized mechanically competent extracellular matrix to withstand load bearing, thus protecting the underlying bones and allowing their near friction-free articulation in the joints. The resident chondrocytes maintain this function throughout life and do so by retaining a stable phenotype that resists hypertrophy and vascular invasion from the bone. This articular phenotype is distinct from the chondrocytes that drive endochondral ossification, which undergo hypertrophy and apoptosis followed by vascularization and bone formation (1). Unfortunately, stem cells used for cartilage repair seem to have a similar fate, making the repair tissue inadequate for normal joint function (2). How the articular chondrocyte avoids this and maintains its specialized phenotype is one of the fundamentally important issues in skeletal biology, yet the mechanisms remain unclear. Recent studies, however, have begun to provide some much needed insights.

Although different growth factors (including transforming growth factor β family members, bone morphogenetic proteins, and fibroblast growth factor family members) are important in skeletal development, there is relatively little evidence that these molecules are endogenously produced in physiologically significant amounts by adult articular cartilage. However, Klinger and colleagues, whose article appears elsewhere in this issue of *Arthritis & Rheumatism* (3), have identified a critical role for secreted matrix protein chondromodulin 1 in stabilizing the chondrocyte phenotype and inhibiting vascular invasion and endochondral ossification in stem cell–mediated articular cartilage repair (3). In their study, using a miniature pig model of cartilage repair, chondromodulin 1 was overexpressed in osteochondral progenitor cells (cells infected with adeno-associated virus vectors carrying chondromodulin 1 complementary DNA [AAV-Chm-1]), or AAV-Chm-1 vectors were directly administered to cartilage defects undergoing microfracture to induce repair. In both cases of chondromodulin 1 treatment, elaboration of a type II collagen–rich matrix was seen at 6 weeks, and most importantly, the tissue resisted calcification and vascular invasion over an extended experimental period (6 months). When progenitor cells were administered to cartilage defects with intact subchondral bone, although little calcification was observed, the repair tissue was fibrocartilaginous, with strong staining for type I collagen and less staining for type II collagen compared to the equivalent chondromodulin 1 treatment.

Chondromodulin 1 has been shown to have antiangiogenic properties (4), but its mechanism of action is far from fully elucidated. Since Klinger and colleagues found no effect of chondromodulin 1 overexpression on *VEGF* mRNA levels, it appears not to inhibit this key angiogenic factor, at least in vitro. However, the situation in vivo is liable to be far more complex, and it will be of great interest to assess the levels of *VEGF* in chondromodulin 1–treated cartilage repair tissue. Klinger and colleagues also suggest that chondromodulin 1 prevents chondrocyte hypertrophy through suppression of type X collagen in cultured osteochondral progenitor cells. This in vitro finding must now be investigated in vivo, and it will also be of great interest to investigate if runt-related transcription factor 2 (RUNX-2) is down-regulated by chondromodulin 1, since this transcription factor plays an important role in endochondral ossification through regulation of type X collagen and induction of *VEGFA* (5,6).

The role of SOX9 in this process should also be investigated further. SOX9 has been shown to be essential for early events in cartilage differentiation (7) and for expression of the cartilage-specific matrix genes in human articular chondrocytes (8). Moreover, mutations in *SOX9* cause the severe skeletal abnormality of campomelic dysplasia (9). Interestingly, however, *SOX9* is greatly down-regulated as chondrocytes undergo hyper-

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trophy in the growth plate during endochondral ossification (10). This raises the question: Is maintenance of *SOX9* expression key to maintenance of the permanent articular cartilage? In a very elegant series of experiments, Hattori and colleagues (11) made some progress in addressing this issue. Through the development and use of mice misexpressing *Sox9* specifically in hypertrophic chondrocytes (under the control of a BAC-Col10a1 promoter), they demonstrated that *Sox9* is an important negative regulator of cartilage vascularization and endochondral ossification (11). Furthermore, they showed by in situ hybridization and real-time polymerase chain reaction that *Vegfa*, *Mmp13*, and osteopontin were all down-regulated in hypertrophic chondrocytes that misexpress *Sox9*, thus indicating that terminal differentiation was inhibited. *Vegfa* was also shown to be negatively regulated by direct binding of *Sox9* to the *Vegfa* promoter.

Although chondromodulin 1 was not investigated in that study, it is very tempting to speculate that SOX9 regulation of chondromodulin 1 plays an important role in maintaining articular cartilage, in particular by inhibiting vascularization. Shukunami and colleagues (12) performed some very informative localization studies of chondromodulin 1 in the developing mouse and chick (12). They found that onset of chondromodulin 1 expression was associated with chondrogenesis during mouse embryonic development, being highly expressed in the avascular regions of the developing cartilage. Chondromodulin 1 strongly colocalized with the main cartilage matrix marker and key *Sox9* target gene, *Col2a1*, in the developing chick wing. Most interestingly, chondromodulin 1 was also detected in the avascular mesenchyme in chick embryos, including the developing heart, and colocalized strongly with *Sox9* in the avascular endocardial cushions that form the valve structures. Indeed, several of the key *Sox9* target genes, which are critical to cartilage function (e.g., *Col2a1*), have also been detected in the heart valves (13).

Recently, it was shown that *Sox9* function is key in regulating normal heart valve function in adult mice, and its reduction (by crossing loxP-flanked *Sox9* with *Col2a1-cre* mice) led to calcification of the heart valves (the main contributor to heart valve malfunction) (14). Ectopic calcification was also observed by direct knockdown of *Sox9* in heart valves in explant experiments; and increased *Runx2* and osteopontin expression was detected. Hence, the parallels with development and maintenance of articular cartilage are most striking, with both tissue types requiring SOX9 levels to be maintained in order to prevent calcification and maintain normal tissue structure and, hence, function.

When one considers the function of the heart valves, these parallels are perhaps not quite so surprising. Similar to articular cartilage, the heart valves must develop a specialized extracellular matrix to resist the harsh mechanical environment in which they function throughout life. For both tissue types, this environment also excludes the possibility of the development of delicate blood vessels; therefore, avascularity is a functional requirement, and chondromodulin 1 seems likely to play a key role in this process.

In human articular chondrocytes, chondromodulin 1 has been shown to be highly SOX9-dependent, and furthermore, chondromodulin 1 expression was shown to be up-regulated in response to hypoxic conditions, which may more closely mimic those found in vivo in the avascular human articular cartilage (15). In fact, this hypoxic up-regulation of chondromodulin 1 in isolated human articular chondrocytes was largely prevented by depletion of SOX9 using RNA interference. Hence, chondromodulin 1 is most likely indirectly up-regulated by hypoxia, i.e., via SOX9, which is up-regulated under hypoxic conditions by hypoxia-inducible factor 2α (8). Whether SOX9 directly binds and regulates chondromodulin 1 is an important unresolved question. However, this pathway provides one plausible mechanism by which this avascular tissue maintains its function throughout life, i.e., the chronic hypoxia in the cartilage helps maintain SOX9 expression levels that prevent vascular invasion (through, e.g., chondromodulin 1 induction) in addition to direct inhibition of terminal differentiation and subsequent calcification of the tissue as occurs, for example, in the murine growth plate when *Sox9* levels greatly decrease prior to hypertrophy (10).

The role of mechanical factors is also likely to be important in the maintenance of articular cartilage. For example, parathyroid hormone–related protein (PTHrP), which is critical to regulation of the growth plate, has been shown to be up-regulated in response to mechanical loading in cultured chondrocytes (16). Furthermore, a role has been suggested for load-induced expression of PTHrP in the maintenance of articular cartilage following experiments in a mouse model of joint loading and unloading (17). Much work remains to be done to investigate the relevant mechanosensitive mechanisms participating in articular cartilage homeostasis and specifically prevention of hypertrophy and vascularization; a fruitful area of future investigation will be the intersection of hypoxia and mechanically regulated pathways. Such insights are needed not only to

further our basic understanding of articular cartilage biology, but also to help develop novel and improved strategies for cartilage repair and treatments for degenerative diseases such as osteoarthritis.

AUTHOR CONTRIBUTIONS

Dr. Murphy drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

REFERENCES

1. Pacifici M, Koyama E, Iwamoto M. Mechanisms of synovial joint and articular cartilage formation: recent advances, but many lingering mysteries. Birth Defects Res C Embryo Today 2005;75: 237–48.
2. Pelttari, K, Steck E, Richter W. The use of mesenchymal stem cells for chondrogenesis. Injury 2008;39 Suppl 1:S58–65.
3. Klinger P, Surmann-Schmitt C, Brem M, Swoboda B, Distler J, Carl HD, et al. Chondromodulin 1 stabilizes the chondrocyte phenotype and inhibits endochondral ossification of porcine cartilage repair tissue. Arthritis Rheum 2011;63:2721–31.
4. Hiraki Y, Inoue H, Iyama K, Kamizono A, Ochiai M, Shukunami C, et al. Identification of chondromodulin I as a novel endothelial cell growth inhibitor: purification and its localization in the avascular zone of epiphyseal cartilage. J Biol Chem 1997;272: 32419–26.
5. Kim IS, Otto F, Zabel B, Mundlos S. Regulation of chondrocyte differentiation by Cbfa1. Mech Dev 1999;80:159–70.
6. Zelzer E, Glotzer DJ, Hartmann C, Thomas D, Fukai N, Soker S, et al. Tissue specific regulation of VEGF expression during bone development requires Cbfa1/Runx2. Mech Dev 2001;106:97–106.
7. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. Nat Genet 1999;22:85–9.
8. Lafont JE, Talma S, Murphy CL. Hypoxia-inducible factor 2α is essential for hypoxic induction of the human articular chondrocyte phenotype. Arthritis Rheum 2007;56:3297–306.
9. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994;372:525–30.
10. Zhao Q, Eberspaecher H, Lefebvre V, de Crombrugghe B. Parallel expression of Sox9 and Col2a1 in cells undergoing chondrogenesis. Dev Dyn 1997;209:377–86.
11. Hattori T, Muller C, Gebhard S, Bauer E, Pausch F, Schlund B, et al. SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. Development 2010;137:901–11.
12. Shukunami C, Takimoto A, Miura S, Nishizaki Y, Hiraki Y. Chondromodulin-I and tenomodulin are differentially expressed in the avascular mesenchyme during mouse and chick development. Cell Tissue Res 2008;332:111–22.
13. Lincoln J, Lange AW, Yutzey KE. Hearts and bones: shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development. Dev Biol 2006;294:292–302.
14. Peacock JD, Levay AK, Gillaspie DB, Tao G, Lincoln J. Reduced Sox9 function promotes heart valve calcification phenotypes in vivo. Circ Res 2010;106:712–9.
15. Lafont JE, Talma S, Hopfgarten C, Murphy CL. Hypoxia promotes the differentiated human articular chondrocyte phenotype through SOX9-dependent and -independent pathways. J Biol Chem 2008;283:4778–86.
16. Tanaka N, Ohno S, Honda K, Tanimoto K, Doi T, Ohno-Nakahara M, et al. Cyclic mechanical strain regulates the PTHrP expression in cultured chondrocytes via activation of the Ca^{2+} channel. J Dent Res 2005;84:64–8.
17. Chen X, Macica CM, Nasiri A, Broadus AE. Regulation of articular chondrocyte proliferation and differentiation by Indian hedgehog and parathyroid hormone–related protein in mice. Arthritis Rheum 2008;58:3788–97.