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Chondrogenesis, Joint Formation, and Articular Cartilage Regeneration

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ABSTRACT

The repair of joint surface defects remains a clinical challenge, as articular cartilage has a limited healing response. Despite this, articular cartilage does have the capacity to grow and remodel extensively during pre- and post-natal development. As such, the elucidation of developmental mechanisms, particularly those in post-natal animals, may shed valuable light on processes that could be harnessed to develop novel approaches for articular cartilage tissue engineering and/or regeneration to treat injuries or degeneration in adult joints. Much has been learned through mouse genetics regarding the embryonic development of joints. This knowledge, as well as the less extensive available information regarding post-natal joint development is reviewed here and discussed in relation to their possible relevance to future directions in cartilage tissue repair and regeneration. J. Cell. Biochem. 107: 383–392, 2009. © 2009 Wiley-Liss, Inc.

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Understanding the biology of articular cartilage and chondrocytes and in particular, their genesis is critical in developing biological approaches to the treatment of cartilage injury and degeneration. Cartilage has a limited healing response to injury. However, it does grow and remodel vigorously during pre- and post-natal development. It is possible that if we can recapitulate such developmental processes, that cartilage repair and/or regeneration could be improved. For example, current approaches for treating cartilage defects such as micro-fractures, abrasions, or chondrocyte implantation require a reparative or regenerative response by the host site to injury. While these approaches provide symptomatic relief, healing is typically incomplete leaving the surrounding tissue predisposed to damage and susceptible to degradative processes that can lead to osteoarthritis (OA). The burgeoning field of stem cell research offers the exciting possibility of applying pleuripotential cells to the site of cartilage injury, rather than simply exposing the endogenous cells to such injurious processes by micro-fracturing or abrading the subchondral bone. Yet, many questions remain regarding how best to promote the formation of true articular cartilage from such cells. In addition, OA-affected chondrocytes are frequently reported to upregulate genes normally detected during embryonic limb formation [Tchetina et al., 2005]. An understanding of the normal roles of such genes, including the Wnts, bone morphogenetic proteins (BMPs), Indian hedgehog (Ihh), and parathyroid hormone related peptide (PTHrP) among many others, is likely to shed light on the pathology of OA.

The challenges posed by articular cartilage injury and degeneration lead us to ask ourselves, how much we truly know about chondrogenesis and arthrogenesis? At present, most of our understanding of joint formation comes from studies on chick and mouse embryos. With the advent of mouse genetics, these studies have yielded abundant information regarding the embryonic functions of many genes critical for joint formation. However, much of articular cartilage and joint development occurs post-natally; and the mechanisms controlling the formation of the osteochondral interface and growth of articular cartilage proper during this period have not yet been extensively studied. A more in-depth understanding of the signals regulating articular cartilage formation and growth would be a critical step towards optimizing the use of bioengineering for future treatment of cartilage injury and OA.

EARLY DEVLOPMENT AND CHONDROGENESIS

The skeleton develops from a densely packed, avascular mesenchyme, called the skeletal blastema. Precursor mesenchymal cells of the blastema can differentiate into cartilage, muscle, or bone cells but when intended for a cartilage lineage, four steps are involved (1) cell migration, (2) aggregation by mesenchymal–epithelial cell interactions, (3) condensation, and (4) chondrocyte differentiation [O'Rahilly and Gardner, 1975; Hall and Miyake, 2000]. Epithelium-derived signals induce mesenchymal chondrification and the

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appearance of cartilaginous nodules. Simultaneously, cells at the periphery become flattened and elongated to form the perichondrium. Chondrocytes then undergo maturation, hypertrophy, and mineralization, which allow cartilage to be replaced with bone via endochondral ossification, a process that requires extracellular matrix remodeling and angiogenesis. These events are controlled by several cellular interactions with the surrounding matrix, growth factors, and other molecular mediators as well as environmental and mechanical signals. Developmental studies in animals have shown that certain homeobox (Hox) transcription factors are critical for early events of limb patterning in undifferentiated mesenchyme, and also regulating the expression of FGF-8 and Sonic hedgehog (Shh) [Kmita et al., 2005], which also modulate proliferation of cells within the condensations of a cartilage anlage [Hall and Miyake, 2000]. In vitro and in vivo studies show that BMP signaling is required for both the formation of precartilaginous condensations and differentiation of cells into chondrocytes [Yoon et al., 2005], whereas condensation growth is regulated by the BMP antagonist, Noggin which inhibits signaling and permits overt differentiation into "chondroblasts" [Pizette and Niswander, 2000; Yoon and Lyons, 2004]. As we discuss in more detail below, key growth factors BMP, FGF, and hedgehog signaling as well as factors that regulate their expressions remain important throughout development, growth, and homeostasis in articular chondrocytes.

JOINT SPECIFICATION AND INTERZONE FORMATION

The morphology of a developing synovial joint and the process of joint cavitation have been described in many classic studies carried out on the limbs of mammalian and avian embryos [Fell, 1925] and recently reviewed in Pacifici et al. [2005]. Within developing condensations, a cartilagenous interzone first appears, specifying the future joint and segmenting a portion of the limb bud. This is followed by development of a three-layered interzone, which consists of two chondrogenic, perichondrium-like layers that cover the opposing surfaces of the cartilage anlagen and are separated by a narrow band of densely packed cellular blastema that remains and forms the interzone "proper." It is within this central interzone that cavitation and development of the joint space eventually ensues. This process involves mechanospatial changes and cell migration facilitated by increasing levels of hyaluronan, synthesized by uridine diphosphoglucose dehydrogenase (UDPGD) and hyaluronan synthase with its cell surface receptor, CD44. The Wnt gene Wnt14 is one of the earliest markers of interzone formation and induces UDPGD and other genes [Drachman and Sokoloff, 1966; Edwards et al., 1994; Hartmann and Tabin, 2001].

A recent study has shown that the cartilaginous interzone consists of committed chondrocytes that have already begun to express COL2A1, but which cease to express matrilin-1 (Mat1) [Hyde et al., 2007]. While it is unclear what the function of Mat1 may be in defining differentiated phenotypes, the sequence of gene expression observed in this study suggests that a divergence in differentiation between articular and "growth" chondrocytes may occur after initial chondrocyte commitment, at the initial stage of joint specification, when the interzone first appears.

These early events in joint formation establish the joint as a center of chondrogenic modulation that provides inhibitory signals at the ends of future bones to balance the vascular ingrowth and ossification that will later occur in the middle of those bones. For example, Wnt14, whose expression is required for the earliest steps of joint specification, acts through a non-canonical (non-β-catenin) pathway, and negatively regulates chondrogenic differentiation at the site of future joints [Hartmann and Tabin, 2001]. The BMP family member, growth and differentiation factor-5 (GDF5, also called cartilage-derived morphogenetic protein-1 or CDMP1) has relatively "pro-chondrogenic" activities, such as mesenchymal cell recruitment and induction of chondrogenic differentiation; and it is also an early marker of joint formation [Francis-West et al., 1996, 1999; Storm and Kingsley, 1999; Coleman and Tuan, 2003]. However, GDF5 and other BMPs require modulation by antagonists such as Noggin and Chordin for normal joint development, as is demonstrated in the fused and overgrown joints of mice lacking these antagonist genes [Brunet et al., 1998; Francis-West et al., 1999].

CHONDROCYTE PROLIFERATION AND MATURATION

Deep to the articular surfaces, endochondral ossification progresses in the developing epiphyseal growth plate, which in prenatal animals (including humans) is continuous with and indistinguishable from the future articular cartilage. In contrast to adult articular cartilage in which cellular proliferation is rarely seen, growth plate chondrocytes proliferate rapidly under the regulation of multiple mitogenic stimuli, which converge on the cyclin D1 gene [Beier, 2005]. Exit from the proliferative state is linked to the onset of maturation, progressing to hypertrophy, apoptosis, and finally, ossification.

As in earlier stages of development, the FGF/BMP axis remains important. The temporal-spatial balance between BMP and FGF ligands and receptors determines the rate of chondrocyte proliferation [Minina et al., 2002; Ornitz, 2005]. During the transition of proliferating chondrocytes to prehypertrophic cells in the growth plate, FGFR3 serves as a master inhibitor of proliferating chondrocytes via phosphorylation of Stat1 transcription factor, which increases expression of the cell cycle inhibitor, p21 [Sahni et al., 1999]. The FGF-18 is the preferred ligand of FGFR3, as shown by FGF-18-deficient mice that have an expanded zone of proliferating chondrocytes similar to that in FGFR3-deficient mice [Liu et al., 2002]. Both FGF-18 and FGF-9 are expressed in perichondrium and periosteum and form a functional gradient in the proximal zone of proliferating chondrocytes, where FGF18 acts via FGFR3 to downregulate proliferation and promoting subsequent maturation [Liu et al., 2002; Ohbayashi et al., 2002]. As the epiphyseal growth plate develops, FGFR3 disappears and FGFR1 expression is upregulated in prehypertrophic and hypertrophic chondrocytes, suggesting a role of FGFR1 in regulating cell survival and differentiation, and possibly cell death [Itoh and Ornitz, 2004].

In the prehypertrophic and hypertrophic zones, both FGF-18 and FGF-9 interact with FGFR1 and regulate vascularization by inducing the expression of the angiogenic vascular endothelial growth factor (VEGF) and its receptor VEGFR1.

Of interest, mature articular chondrocytes express primarily FGFR1 and amongst the FGF family, the most studied in articular chondrocytes is FGF-2, or basic FGF, which is a potent mitogen for adult articular chondrocytes [Trippel, 2004]. Early studies suggested that low concentrations of FGF-2 could stimulate chondrocyte mitogenesis and proteoglycan synthesis whereas high concentrations might have inhibitory effects [Sah et al., 1994]. Recent studies that showed FGF-2, stored in adult cartilage matrix, is released after mechanical injury or joint loading suggests a mechanism for modulating chondrocyte proliferation and anabolic activity [Vincent et al., 2002, 2004]. FGF-2 can also inhibit the anabolic activities of IGF-1 and OP-1 in vitro [Loeser et al., 2005]. Both FGF-9 and FGF-18 increase matrix synthesis by mature chondrocytes [Weksler et al., 1999; Ellsworth et al., 2002; Shimoaka et al., 2002; Au et al., 2004]. An interesting finding in a recent study demonstrated that FGF-18 promotes cartilage repair in a rat meniscal tear model of OA [Moore et al., 2005], indicating that it is another potentially important factor in neo-cartilage formation.

The interaction of Ihh with PTHrP is also critical in controlling the rate of chondrocyte proliferation and maturation. Classic studies have shown the presence of a negative feedback mechanism between the prehypertrophic cells, which express Ihh and the periarticular resting zone chondrocytes, which express PTHrP in response to the direct and indirect effects of Ihh [Karaplis et al., 1994; Lanske et al., 1996; Vortkamp et al., 1996; Chung et al., 2001; Kronenberg, 2006]. The proliferating and prehypertrophic cells, in turn, respond to PTHrP by proliferating, which inhibits the progression to maturation and the additional expression of Ihh. Studies in mouse genetics have established the important role of these genes and their receptors in the organization and maintenance of the architecture of the epiphyseal growth plate. Some evidence indicates that Ihh can act independently of PTHrP on periarticular chondrocytes and stimulate the differentiation of columnar chondrocytes in the proliferative zone [Kobayashi et al., 2005]. In addition to PTHrP induction, Ihh signaling can also induce BMP-4, another mitogenic factor, and this is dependent on mechanical stimulation [Wu et al., 2001]. One reason that Ihh signaling is modulated by mechanical stimulation is the association of the Ihh receptor Ptc with cilia. However, in comparisons of Ihh- and Kif3a-deficient mice, chondrogenesis differs significantly, indicating that Ihh actions may not depend solely on molecular association with cilia [Kolpakova-Hart et al., 2007; Koyama et al., 2007].

In the articular cartilage PTHrP has been reported to inhibit mineralization that is associated with OA [Terkeltaub et al., 1998]. In addition, our recent studies indicate that the PTHrP/Ihh axis is still active post-natally, potentially regulating mineralization by chondrocytes at the osteochondral interface of immature joints [Jiang et al., 2007]. An interesting study in fetal lamb showed that at this stage, articular/epiphyseal cartilage injuries heal completely without any scar or fibrocartilage formation evident after birth [Namba et al., 1998]. The elucidation of the signals that orchestrate the embryonic repair response may provide future insights into how mature articular cartilage might be healed.

ENDOCHONDRAL OSSIFICATION AND FORMATION OF THE OSTEOCHONDRAL INTERFACE

At birth, the articular cartilage of many joints in humans and mice are still indistinguishable from the epiphyseal growth plate. Soon after birth, however, a secondary ossification center (SOC) appears within the epiphyseal cartilage, dividing it into the future metaphyseal growth plate proximally and the articular surface distally. In the metaphyseal growth plate, local chondrocyte regulation through factors such as PTHrP, Ihh, FGFs, BMPs, and VEGF persist, but influences from the endocrine hormones (particularly thyroid hormone and estrogen) become important. The gradual thinning of cartilage distal to the SOC would suggest that a similar process of endochondral ossification occurs in the subchondral region around joints as during post-natal growth and development in the metaphyseal growth plate. On the other hand, the process at the osteochondral interface has to be significantly different from that in the growth plate, for around the joint, ossification must be halted in a manner to leave cartilage at the joint surfaces. Furthermore, the appearance of the histological tidemark, an indication of the formation of the zone of calcified cartilage, is not necessarily temporally coordinated with growth plate closure, suggesting that these processes are regulated by divergent mechanisms. Finally, the quality of the bone of the subchondral plate is different in structure and mineral density compared to the metaphyseal trabecular bone, again supporting that subtly different mechanisms may control ossification in these areas.

Recent studies have shown that formation of the SOC depends on the matrix degrading activity of MT-MMP1 (MMP14) [Holmbeck et al., 1999]. In particular MMP14 is required for the normal invasion of blood vessels into the epiphyseal cartilage that occurs early in the process of SOC formation. The expression of MMP14 is also evident at bone/tendon junctions, suggesting a particularly important role for this molecule in interface tissues.

In the epiphyseal growth plate of the embryo as well as the metaphyseal growth plate of the post-natal animal, endochondral ossification involves terminal differentiation of chondrocytes to hypertrophy, calcification of cartilage matrix, vascular invasion, and ossification [Ferguson et al., 1998; Colnot and Helms, 2001; Ballock and O'Keefe, 2003; Provot and Schipani, 2005]. During chondrocyte hypertrophy, there is notable increase in cell size, up to 20-fold of its initial resting size. At this time they begin to express markers of hypertrophic chondrocytes such as type X collagen (COL10a1) and alkaline phosphatase [St-Jacques et al., 1999]. The runt-related transcription factor 2 (Runx2) [Komori et al., 1997; Otto et al., 1997; Ferguson et al., 1998; Enomoto et al., 2000; Colnot, 2005] and BMP-induced Smad1 [Enomoto et al., 2000; Leboy et al., 2001; Zheng et al., 2003] are critical positive regulators of hypertrophy and ossification through direct upregulation of COL10a1, MMP-13, and other genes. The activities of MMP-13 and other MMPs such as MMP-9 are required for ECM remodeling, the rate-limiting step for chondrocyte hypertrophy, angiogenesis and

the subsequent replacement of mineralized cartilage by bone through the invasion of osteoclasts and osteoblasts [Ortega et al., 2004].

Ossification requires angiogenesis; and the perichondrium and hypertrophic zones are invaded by blood vessels, a process regulated by VEGF [Zelzer and Olsen, 2005] and its receptors. As mentioned earlier, Ihh is expressed in prehypertrophic chondrocytes and during ossification Ihh synchronizes skeletal angiogenesis with perichondrial maturation [Chung et al., 2001]. The expression of VEGF is also regulated by hypoxia inducible factor 1α (HIF1α), which is responsive to tissue oxygen tension [Schipani et al., 2001]. Protein activation of several VEGFs is dependent, in part, on release from the ECM by MMP-9 [Ortega et al., 2004]. Upregulation of VEGF in OA-affected chondrocytes and the appearance of vessels breaking through the subchondral plate in OA-affected cartilage may be pathological. However, developing articular cartilage is normally vascular, particularly in the deeper zones. Whether these vessels are also controlled by VEGF and more important, whether they contribute to the growth or remodeling of cartilage prior to skeletal maturity are interesting questions for future investigations.

Chondrocyte hypertrophy during endochondral ossification is coordinated by transcriptional regulators that inhibit or enhance the function of Runx2 [Komori, 2005]. We have recently reported that GADD45β, which has been implicated in the stress response and cell survival during terminal differentiation of different cell types, is prominent in the early response gene to BMP-2 through a Smad1/Runx2-dependent pathway and acts as a survival factor to maintain Mmp13 and Col10a1 expression in hypertrophic chondrocytes [Ijiri et al., 2005]. The homeodomain protein Nkx3.2, which is an early BMP-induced signal required at the onset of chondrogenesis is also a direct transcriptional repressor of Runx2 promoter activity [Lengner et al., 2002]. Another such factor is, ERG/C-1-1, a transcription factor of the ETS family and is specific to articular chondrocytes [Iwamoto et al., 2000, 2005, 2007]. The SRY related transcription factor Sox9, an early marker of chondrocytes [Zhou et al., 2006], the bHLH factor Twist [Bialek et al., 2004], and PTHrP [Iwamoto et al., 2003] can all also oppose Runx2 activity. In contrast, cooperation of the Groucho homologue Grg5 or the leucine zipper protein ATF4 with Runx2 promotes chondrocyte maturation [Wang et al., 2004] or osteoblast differentiation [Xiao et al., 2005], respectively. A recent study has shown that haplo-insufficiency of Runx2 is protective against experimental OA, implicating the inhibition of Runx2 as an important mechanism for articular cartilage homeostasis [Kamekura et al., 2006]. As such, future investigations into the functions of such factors as Nkx3.2, Sox9, Twist1, and GADD45β of Runx regulation are of particular interest in understanding normal articular cartilage growth, homeostasis, and OA.

Certain hormones have been shown to play particularly important regulatory roles in controlling endochondral ossification of the post-natal metaphyseal growth plate [van der Eerden et al., 2002]. This is in contrast to prenatal development where hormone effects are less important than those of the paracrine growth factors that we have discussed so far. An example is estrogen, whose levels must neither be too low nor too high to ensure normal growth plate chondrocyte maturation and long bone growth [Takano et al., 2008]. Studies in knock out mice indicate that estrogen controls growth plate closure; but whether it controls the formation of the tidemark was not examined [Vidal et al., 2000; Chagin et al., 2004]. Studies in OA suggest that estrogen may be protective. Further studies are required to understand whether the patient's estrogen status, or its exogenous use might be relevant in engineering or regenerating new cartilage tissue.

Our current studies show the persistence of PTHrP and Ihh, expressed in a zone-specific manner in post-natal developing joints (see Fig. 1). However, the distribution and morphology of the subpopulations are distinct from that which is seen in the growth plate, suggesting the presence of distinct regulatory mechanisms. Of note, Ihh staining is particularly strong in chondrocytes at the articular surface and may suggest a role in resisting hypertrophy, mineralization, and/or ossification of chondrocytes. Also, of particular interest, the hypertrophic chondrocytes underlying the future joint surface are much larger than those in the metaphyseal growth plate and appear to be much less organized than those in the metaphyseal growth plate. To elucidate how ossification in this zone of chondrocyte hypertrophy differs from that of the metaphyseal growth plate is likely to be important. For example, such knowledge may shed light on possible methods to prevent bony overgrowth from the underlying bone after cartilage repair using micro-fracture or other techniques that activate the subchondral bone. Understanding the biology underlying the development of the

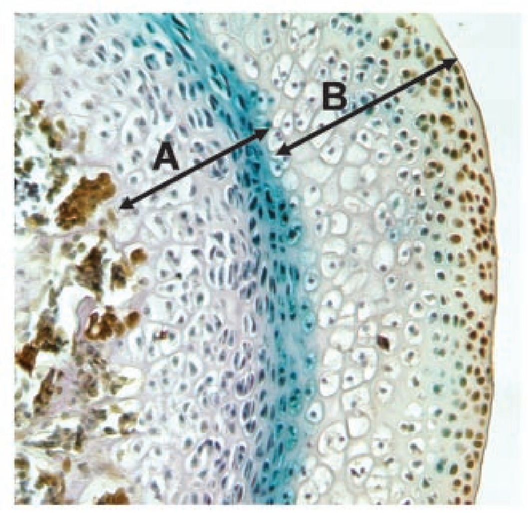

Fig. 1. Presence of Ihh and expression of PTHrP in the femoral head of a 3-week-old mouse. The Ihh was visualized by immunohistochemistry in mice expressing β-galactosidase under the control of PTHrP. Two zones of maturation and endochondral ossification are apparent. A growth plate [A] is evident with PTHrP-expressing (blue) cells overlying proliferative columnar cells, hypertrophic cells, and new bone in the expected organization. Distally, the articular surface [B] is populated with Ihh-positive (brown) cells overlying a subpopulation of PTHrP-expressing (blue, β-galactosidase-positive cells). The hypertrophic cells deep to the articular surface are likely destined to undergo endochondral ossification, becoming the subchondral bone plate. However, they are larger and in a relatively random organization compared to those in the growth plate, suggesting that subtly different mechanisms may orchestrate its ossification.

tidemark may also provide insight into design strategies for creating mechanically sound osteochondral interfaces between engineered cartilage tissue and the subchondral bone. Finally, hypertrophy-like processes observed in OA-affected chondrocytes may be treatable, if we can understand their normal function in articular cartilage.

ADULT ARTICULAR CARTILAGE AND ITS ORGANIZATION

The zonal architecture of articular cartilage is not only a developmental phenomenon, but also a characteristic that is evident in adult articular cartilage, after the formation of the tidemark and the zone of calcified cartilage. Chondrocytes that populate the articular cartilage comprise four distinct regions: (1) the superficial tangential (or gliding) zone, (2) the middle (or transitional) zone, (3) the deep (or radial) zone, and (4) the calcified cartilage zone, which is located immediately below the tidemark and above the subchondral bone [Hunziker et al., 1997; Poole et al., 2001]. In the superficial zone, chondrocytes are small and flattened, and their surrounding matrix is composed of thin collagen fibrils in a tangential array, composed of a high concentration of decorin and a low concentration of aggrecan. The middle zone, comprising 40–60% of the cartilage weight, consists of round chondrocytes surrounded by radial bundles of thick collagen fibrils. In the deep zone, chondrocytes are frequently grouped into columns or clusters with thick collagen bundles arranged in a radial-like manner. From the surface to the deep zone, the cell volume increases, progressively reducing the cell density and increasing the proportion of proteoglycan relative to collagen content. The "tidemark" serves as an important barrier between the uncalcified articular cartilage and the subchondral bone [Lane et al., 1977]. Differences in the subpopulations of articular chondrocytes: superficial, middle, and deep zone chondrocytes (SZC, MZC, and DZC) have largely been thought to arise as a consequence of their unique mechanical milieu [Hamerman et al., 1970; Hunziker et al., 1997]. A recent in vitro study by Vanderploeg et al. [2008] provides new evidence for this concept, showing that DZCs, which are normally under compressive load, alter the profile of their matrix production to that which is more like SZC, when stimulated with tensile load to mimic a mechanical environment more like the joint surface. In vitro studies of isolated SZC and DZC indicate that they maintain the zonal differences in metabolic activities that reflect the zonal differences in matrix compositions of the original tissue [Zanetti et al., 1985; Aydelotte and Kuettner, 1988; Aydelotte et al., 1988; Siczkowski and Watt, 1990; Waldman et al., 2003; Darling and Athanasiou, 2005; Hidaka et al., 2006; Cheng et al., 2007].

How does the organization of articular cartilage develop and how may it be recapitulated in situations that require cartilage repair or regeneration? Recent studies offer clues, although much remains to be uncovered. Measuring rates of bone growth proximal to developing joints in the rabbit, [Hunziker et al., 2007] found evidence that most of the cartilage present at the ends of the bone at birth is replaced by bone and that the cartilage of the articular surfaces is formed anew over the first 3 months of post-natal life. While these observations do not refute the concept that ossification and mineralization must be arrested to form a normal osteochondral interface, they suggest that articular cartilage proper may not represent the remnants of the epiphyseal growth plate, but tissue that is added to the articular surface by appositional growth. Indeed, classic studies in the growing rabbit by Mankin [1964] and Mankin and Orlic [1964] identified two zones of articular chondrocyte proliferation—one near the developing osteochondral interface and a second at the articular surface, where appositional tissue growth might occur. In support of the possibility of appositional growth at the cartilage surface, several investigators have reported the presence of stem cells at the articular surface of neonatal joints [Hayes et al., 2001, 2007; Dowthwaite et al., 2004; Hattori et al., 2007]. In particular Archer and Dowthwaite [Dowthwaite et al., 2004] used immature bovine and mouse tissues to isolate Notch1-selected chondrocytes from the superficial zone of developing articular cartilage and showed that they have stem cell-like qualities, including significant plasticity in differentiation pathways, and a cloning capacity. Fibronectin, a common adhesion molecule highly expressed in epidermal stem cells [Jones and Watt, 1993] is also seen in the chondrocytes of articular surface of a developing articular cartilage with significantly higher binding affinity to SZ chondrocytes than to MZ or DZ chondrocytes, especially to the Notch1-selected cells of the superficial zone. Inhibition studies suggest that Notch-1 might play a role in chondrocyte proliferation [Karlsson et al., 2008].

Based on these findings, several groups have attempted to engineer cartilage using SZ and DZ [Han et al., 2003; Kim et al., 2003; Klein et al., 2003; Sharma et al., 2007]. While these studies did result in the formation of a striated tissue that mimicked normal articular cartilage morphologically, no evidence of appositional growth was sought or observed. Clearly, additional investigation is required to attempt to recapitulate appositional cartilage growth and the formation of the normal articular cartilage architecture in the context of cartilage repair and/or regeneration.

Other studies suggest that articular cartilage stem cells may reside in the synovial tissue, which also arises from the interzone. Hunziker [2001] have shown that cells can be induced to migrate from the synovium overlying the articular surface to the site of lesion and under the influence of growth factors can fill the defect with a repair tissue. Others have shown that pleuripotent cells with chondrogenic capacity can be isolated from the synovium [Sakaguchi et al., 2005; Shirasawa et al., 2006]. Such findings prompt a deeper understanding of the development of the zonal architecture of articular cartilage, and in particular the possibility that the superficial zone and/or synovium may be sources of articular cartilage-specific cells with stem-like characteristics.

CHONDROGENIC MEDIATORS OF THE ARTICULAR CARTILAGE

Several factors that regulate chondrocyte behavior in the growth plate remain important mediators of the metabolism of adult articular chondrocytes, although any given growth factor usually exerts distinct effects in growth plate versus articular chondrocytes.

It remains unclear whether these differences arise from distinct stages of differentiation in the two types of chondrocytes or from their distinct origins.

Knockout studies causing defective TGF-β signaling have excluded it as a potent inducer of chondrocyte proliferation. The knockout animal models develop premature joint degeneration suggesting that endogenous activity is necessary for normal matrix homeostasis [Serra et al., 1997; Yang et al., 2001]. Findings that IL-1 distinctly regulates inhibitory Smads [Kaiser et al., 2004] and decreases TGFβ signaling also support the protective effect of endogenous TGFβ during OA progression [Blaney Davidson et al., 2006]. Furthermore, administration of agents that block TGFβ activity, such as the soluble form of TGFβRII, inhibitory SMADs, or the physiological antagonist, latency-associated peptide-1 (LAP-1), increases proteoglycan loss and cartilage damage in an experimental model of OA [Scharstuhl et al., 2003]. However, a recent finding that TGFβ induces expression of ADAMTS-4 in primary human chondrocytes and promotes the degradation of aggrecan suggests that it may be involved in normal turnover of proteoglycans in mature cartilage [Moulharat et al., 2004]. Indeed, effects of exogenous TGF on chondrocyte matrix metabolism are variable.

Several BMPs including BMP-2, 4, 6, 7, 9, 13 (also known as CDMP-2) and 15 (CDMP1) can enhance the synthesis of type II collagen and aggrecan by articular chondrocytes in vitro [Chubinskaya and Kuettner, 2003; Grunder et al., 2004]. In addition, BMP-2, -7, and -9 counter many of the catabolic responses induced by IL-1 including induction of MMP-1 and MMP-13, down-regulation of TIMP expression, and down-regulation of proteoglycan synthesis in primary human articular chondrocytes [Chubinskaya and Kuettner, 2003]. BMP-7 (also known as osteogenic protein-1 or OP-1) is expressed in mature articular cartilage and is possibly the strongest anabolic stimulus for adult chondrocytes in vitro, because it increases aggrecan and type II collagen synthesis more strongly than IGF-I [Chubinskaya and Kuettner, 2003]. A requirement for signaling of BMPs and related factors such as GDFs has been shown in studies resulting in premature joint degeneration as a result of BMPR1A deletion in the joint [Rountree et al., 2004]. The necessity for BMP signaling for normal cartilage matrix homeostasis has also been corroborated in studies in human cartilage explants [Merrihew et al., 2003; Soder et al., 2005].

The pleiotropic effects of BMPs are well known; and a series of recent studies suggest that whether BMPs support matrix synthesis or promote chondrocyte differentiation depends on the differentiation state of the chondrocyte, in part through intra-cellular mechanisms balancing TGFβ and BMP signaling. Specifically, Zuscik et al. [2004] showed that treatment of chick articular chondrocytes with 5-azacytidine (a pro-differentiating agent used for cancer therapy) induced a maturational response in the cells upon BMP-2 stimulation, suggesting that articular chondrocytes may be in a suspended state of differentiation that can be reactivated to undergo the endochondral ossification pathway. Furthermore, this group showed that the presence of Smurfs, which ubiquitinate and thus control the relative availability of Smads, the intra-cellular signaling molecules downstream of TGFβ and BMPs, was critical to this change in BMP responsiveness. Recently, we have shown that such differences in responsiveness to BMPs also characterize the difference in the superficial versus deep zone chondrocyte phenotypes, with BMP-2 inducing matrix synthesis but not hypertrophy in SZC, while inducing hypertrophy without increasing matrix synthesis in DZC [Cheng et al., 2007]. Previously we have implanted BMP7 over expressing chondrocytes into experimental cartilage defects in the horse [Hidaka et al., 2003]. While this improved early repair, morphogenetic effects were not sufficient to form tissue with the appearance and function of normal cartilage within the repair tissue. Ours and many other studies point to the need for continued research towards the goal of engineering and/or regenerating true hyaline cartilage for the purpose of joint injury repair or degeneration.

CONCLUSION

The molecular and cellular mechanisms controlling chondrogenesis and joint formation offer a wealth of knowledge about articular chondrocyte behavior and how it is regulated. Certainly, such information can be utilized in designing novel bioengineering approaches for articular cartilage repair and regeneration; but further investigations are clearly still required. The studies reviewed in this article indicate that articular cartilage formation requires a balance between negative and positive chondrogenic (or maturational) factors particularly in the initial differentiation of articular chondrocytes, but also during post-natal development and homeostasis in the mature joint. In the earliest phase of articular chondrogenesis, Wnt14 may be particularly important, and later the balance between FGF and BMPs (as well as BMP antagonists), between PTHrP and Ihh. Also critical to this balance are the regulators of Runx2, which may control chondrocyte hypertrophy in articular chondrocytes, much as in the growth plate. These regulators, including ERG/C1-1, Sox9, Twist1, and GADD45β amongst others are likely to be particularly interesting for future study. Duplicating the balance between negative and positive chondrogenic (or maturational) factors will likely be required to engineer neo-cartilage that is not subject to endochondral ossification when implanted onto subchondral bone. Furthermore, such a balance, in concert with mechanical stresses, may contribute to the normal zonal organization of cartilage. As early studies suggest, re-capitulating this normal architecture may help produce engineered neo-cartilage that can function mechanically in a manner similar to normal cartilage. Finally, the study of endochondral ossification at the osteochondral interface is also likely to be of significant importance. Early morphologic studies indicate a process slightly different to that occurring in the metaphyseal growth plate. These differences may shed light not only on how maturation and ossification can be prevented in the setting of cartilage repair, but also how vascular invasion through the calcified cartilage may be treated or prevented in the setting of OA. Although the intrinsic response of articular cartilage to injury is minimal, its capacities for growth and remodeling during post-natal growth and development are significant. Elucidating these mechanisms may lead to the development of novel biological approaches for

improving articular cartilage tissue repair and/or regeneration in the treatment of injury and/or degeneration.

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