Review

Molecular regulation of articular chondrocyte function and its significance in osteoarthritis

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Summary. Osteoarthritis (OA) is the most common form of joint disease. Histopathologically, OA is characterized by a progressive loss of articular cartilage, osteophyte formation, thickening of subchondral bone, and subchondral cyst formation. All current therapies are aimed at symptomatic control and have limited impacts on impeding or reversing the histopathologic progression to advanced OA. Previous studies have shown that overexpression of matrix-degrading proteinases and proinflammatory cytokines is associated with osteoarthritic cartilage degradation. However, clinical trials applying an inhibitor of proteinases or proinflammatory cytokines have been unsuccessful. A more sophisticated understanding of the regulatory mechanisms that control the function of articular chondrocytes is paramount to developing effective treatments. Since multiple catabolic factors and pathological chondrocyte hypertrophy are involved in the development of OA, it is important to identify which upstream factors regulate the expression of catabolic molecules and/or chondrocyte hypertrophy in articular cartilage. This review summarizes the current studies on the molecular regulation, with a main focus on transcriptional regulation, of the function of adult articular chondrocytes and its significance in the pathogenesis and treatment of OA. Recent studies have discovered that transcription factor Nfat1 may play an important role in maintaining the physiological function of adult articular chondrocytes. Nfat1-deficient mice exhibit normal skeletal development but display most of the features of human OA as adults, including chondrocyte hypertrophy with overexpression of specific matrix-degrading proteinases and proinflammatory cytokines in adult articular cartilage. ß-catenin transcriptional signaling in articular chondrocytes may also be involved in the pathogenesis of OA. Activation of ß-catenin leads to OA-like phenotypes with overexpression of specific matrix-degrading proteinases in articular cartilage of adult mice. These and other regulatory mechanisms described in this review may provide new insights into the pathogenesis of OA and the development of novel therapeutic targets for the treatment of OA.

Key words: Articular chondrocytes, Osteoarthritis, Molecular regulation, Transcription factor, Growth factor

Introduction

Osteoarthritis (OA) is the most common form of joint disease and the major cause of chronic disability in middle-aged and older individuals. Histopathologically, OA is characterized by a progressive loss of articular cartilage, osteophyte formation, thickening of subchondral bone, and subchondral cyst formation. OA can affect any synovial joints, but idiopathic OA is common in the knee, hip, hand, foot, and spine joints. Although idiopathic OA rarely occurs in the ankle, wrist, elbow, and shoulder, the risk of post-traumatic OA in these joints appears as great as in the hand, knee, hip, and foot joints (Buckwalter and Martin, 2006). Recent surveys by the United States (US) National Arthritis Data Workgroup estimated that among US adults, nearly 27 million have clinical OA (Lawrence et al., 2008). Surveys from the World Health Organization estimated that OA affects 9.6% of men and 18% of women aged

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>60 years (Woolf and Pfleger, 2003). As the population continues to age and battles to control obesity, the numbers of those afflicted with OA will continue to grow. From an economic standpoint, the estimated annual cost of OA in the United States could be anywhere from 60 billion to 89 billion dollars (Buckwalter and Martin, 2006; Bitton, 2009). Particularly in a period of increasing scrutiny of health care costs and expenditures, one can appreciate the magnitude of impact attributed to OA.

Many treatments currently exist to target symptomatic control of OA, including pharmacologic therapy, local joint injection, and surgical interventions. Pharmaceuticals such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen aim to control inflammation and pain by blocking potent inflammatory cytokine pathways. Joint injections including glucocorticoids and hyaluranan-based formulations attempt to control inflammatory mediators locally and improve the glucosaminoglycan concentration within the joint space. Surgical procedures such as debridement, microfracture, osteochondral autografting, and autologous chondrocyte transplantation are currently employed to stimulate articular cartilage repair and prevent or delay the need for joint replacement. However, all current therapies are aimed at symptomatic control and have limited impact on impeding or reversing the histopathologic progression to advanced OA. The critical barriers to progress in OA therapy are that: a) molecular and cellular mechanisms for the development of OA, especially the mechanisms that control the activity of adult articular chondrocytes, remain unclear, and b) the biomechanical etiopathogenesis of OA is insufficiently recognized. Therefore, a more sophisticated understanding of the regulatory mechanisms involved in the development of OA is paramount to developing effective treatments.

Within the last decade or so, many exciting discoveries have been made regarding the molecular regulation of bone, cartilage, and joint development. This review does not attempt to cover all of the reported potential regulators of cartilage, bone and joint formation. Rather, we will focus on recent findings and our current knowledge about the molecular regulation, especially transcriptional regulation, of the maintenance of adult articular chondrocyte activity and its significance in pathogenesis and treatment of OA. We will also address current therapeutic modes and challenges, and finally discuss potential novel targets, especially specific transcription factors, for therapeutic intervention.

Regulation of chondrocyte differentiation and joint development

Since many governing factors that are critical for skeletal development have also been found to have a significant involvement in chondrocyte homeostasis and pathophysiology of OA, it is necessary to discuss the regulatory mechanisms of chondrocyte differentiation and joint formation. Both the chondrocyte (cartilage-forming cell) and osteoblast (bone-forming cell) are derived from a common mesenchymal stem cell referred to as the osteochondroprogenitor cell (Eames et al., 2004). Bone formation pathways include both endochondral ossification and intramembranous ossification. Endochondral ossification is of particular interest because it is responsible for the formation of long bones and is also involved in the development of OA.

Limb development first begins with the formation of mesenchymal condensations and the subsequent formation of a surrounding cartilaginous envelope called the perichondrium. These cells proceed to form the anlagen, or cartilaginous template of each bone. These cartilage cells undergo proliferation, differentiation to functional chondrocytes, hypertrophic differentiation, apoptosis, calcification of cartilage matrix, invasion of cartilage tissue by capillaries and chondroclasts (osteoclasts), and eventual resorption and replacement by newly formed bone. This process of bone formation is called endochondral ossification. The primary ossification center in the diaphysis of long bones is formed through endochondral ossification. A similar sequence of events occurs in the growth plate leading to rapid growth of the skeleton. At the end of each long bone an interzone and secondary ossification center develop. The interzone is the first sign of joint development at each future joint location, which consists of closely associated mesenchymal cells. Articular chondrocytes may derive from a subset of interzone cells, especially from the intermediate layer. Physiological separation of the adjacent skeletal elements occurs with further development, which involves a process of cavitations within the interzone that leads to formation of a liquid-filled synovial space. Morphological and cyto-differentiation processes extending over developmental time eventually lead to maturation of the joint in which the proximal and distal ends acquire their reciprocal and interlocking shapes. The formation of hyaline articular cartilage and other joint-specific tissues makes the joint fully capable of providing its physiologic roles through life (Mitrovic, 1978; Pacifici et al., 2005). Factors that may regulate chondrocyte differentiation and joint formation are discussed below.

Sox9

The transcription factor Sox9 is a member of the high-mobility-group (HMG) and appears to be an essential transcription factor driving chondrogenesis during development and growth (Bi et al., 1999). The importance of Sox9 in commitment to the chondrocyte lineage has been demonstrated by gain- or loss-of function studies both *in vitro* and *in vivo*. Sox9 is expressed predominantly by mesenchymal progenitor cells and proliferating chondrocytes, but is not found in

hypertrophic chondrocytes or osteoblasts (Zou et al., 2006). Transcription factor Sox9 is critical for the differentiation of mesenchymal progenitor cells into chondrocytes during cartilage morphogenesis. Prechondrocytic mesenchymal cells lacking Sox9 are unable to differentiate into chondrocytes (Bi et al., 1999). Joint formation is defective in Sox9-deficient mouse embryos (Akiyama et al., 2002). Two other members of the Sox family, L-Sox5 and Sox6 may also be essential for cartilage formation (Smits et al., 2001). Sox9 can upregulate the expression of chondrocyte-specific cell markers, including type-II collagen (collagen-2), collagen-11, and aggrecan genes by binding to their enhancer sequences (Evangelou et al., 2009). Sox9 also acts cooperatively with L-Sox5 and L-Sox6, which are present after cell condensation during chondrocyte differentiation, to activate collagen-2 and aggrecan genes (Zou et al., 2006; Lefebvre et al., 1998; Leung et al., 1998). In addition to its importance in early chondrogenesis, Sox9 may also be important for crucial stages of late endochondral ossification.

Multiple groups have reported that the Sox trio (Sox 5, Sox6, Sox9) inhibit terminal stages of chondrocyte differentiation (Ikeda et al., 2004; Saito et al., 2007); however, the precise underlying regulatory mechanism remains unclear. Recently, Amano et al. demonstrated that the Sox trio inhibited chondrocyte maturation and calcification by up-regulating parathyroid hormone related protein (PTHrP) (Amano et al., 2009). The anabolic effects of insulin-like growth factor-1 (IGF-1), bone morphogenic protein-2 (BMP-2), and fibroblast growth factor-2 (FGF-2) on developing chondrocytes also appear to be mediated, in part, by Sox9 (Lefebvre et al., 1998; Leung et al., 1998; Zehentner et al., 1999; Kolettas et al., 2001; Goldring et al., 2008).

Runx2

Runx2 (also called Cbfa1, Osf2, or AML3) is a member of the Runt family of transcription factors and has been identified as a transcription factor common to the osteogenic cell lineage, as well as that of replacement cartilage (Komori et al., 1999; Takeda et al., 2001; Coffman, 2003). Runx2 expression is often associated with expression of gene products common to the bone phenotype, including collagen-1 and osteocalcin (Ducy et al., 1997). Komori et al. demonstrated the critical involvement of Runx2 in osteogenesis. Runx2$^{-/-}$ mice died shortly after birth and exhibited a cartilaginous skeleton completely void of intramembranous and endochondral ossification due to the maturational arrest of osteoblasts (Komori et al., 1997). Histologic analyses of Runx2$^{-/-}$ mice have also demonstrated delayed maturation of chondrocytes, indicating Runx2's involvement in both osteogenesis and chondrogenesis (Inada et al., 1999). Another study demonstrated the ability of Runx2 overexpression to change permanent cartilage (e.g., articular cartilage) to temporal cartilage (e.g., growth plate cartilage) (Eames

et al., 2004). During endochondral ossification, Runx2 is expressed mainly in the prehypertrophic and, to a lesser extent, in the late hypertrophic chondrocyte (Takeda et al., 2001). Its expression coincides with Indian hedgehog (Ihh), collagen-10, and BMP-6. Matrix metalloproteinase-13 (MMP-13), which is expressed by terminal hypertrophic chondrocytes, is a downstream target of Runx2 (Hess et al., 2001).

c-Maf

Transcription factor c-Maf, a member of the basic leucine zipper (bZIP) superfamily, is required for normal chondrocyte differentiation during endochondral bone formation. C-Maf is expressed in hypertrophic chondrocytes during fetal development. There is an initial decrease in the number of mature hypertrophic chondrocytes in c-maf-null mouse tibiae, with decreased expression domains of collagen-10 and osteopontin (OPN), markers of hypertrophic and terminal hypertrophic chondrocytes, respectively. However, terminal chondrocytes, which express OPN and MMP13, appear later and persist for a longer period of time in *c-Maf$^{-/-}$* fetuses than in control littermates, resulting in expanded chondrocyte maturation zones and a delay in endochondral ossification. These results suggest that c-Maf may facilitate both the initiation of terminal differentiation and the completion of the chondrocyte differentiation program (MacLean et al., 2003).

Wnt/ß-catenin

In the canonical Wnt signaling pathway, Wnts bind the transmembrane Frizzled receptor (Frz) family and co-receptors LRP5/6. Frz receptor activation recruits the cytoplasmic bridging molecule Dishevelled (Dsh) which inhibits glycogen synthase kinase-3ß (GSK-3). This interaction prevents GSK-3 from phosphorylating ß-catenin and avoiding degradation of ß-catenin, allowing ß-catenin to accumulate in the cytoplasm and translocate to the nucleus as a co-transcriptional activator with lymphoid enhancing factor 1/T cell-specific transcription factor (LEF/TCF) at specific DNA binding sites to activate downstream genes (Zou et al., 2006; Deng et al., 2008). Thus, ß-catenin is a key mediator of the canonical Wnt signaling. Any mutation that modulates the expression of the involved proteins can alter the effects of the canonical Wnt signaling. Non-canonical Wnt signaling pathway, which does not involve ß-catenin (Logan and Nusse, 2004), is probably best studied in Drosophila and its function is not covered in this review. The canonical Wnt signaling pathway plays an important role in embryonic morphogenesis during skeletal development. This signaling pathway stimulates the commitment of mesenchymal stem cells to the preosteoblast and mature osteoblast phenotype, and significantly blocks chondrocyte differentiation (Zou et al., 2006). Activation of the canonical Wnt signaling cascade during development in the limb bud and growth

plate chondrocytes stimulates chondrocyte hypertrophy, calcification, and expression of MMPs and vascular endothelial growth factor (VEGF) (Tamamura et al., 2005; Day et al., 2005; Kawaguchi, 2009). Wnt-14 overexpression in chick limb mesenchymal micromass cultures causes a severe inhibition of chondrogenesis. Wnt-14 is necessary for joint formation since it might maintain the mesenchymal nature of the interzone by preventing chondrogenesis. In addition to Wnt-14, the interzone expresses Wnt-4, Wnt-16, and ß-catenin. Conditional ablation of ß-catenin in chondrocytes leads to the absence of joints. Ectopic expression of activated ß-catenin or Wnt-14 in chondrocytes led to ectopic expression of joint markers (Hartmann and Tabin, 2001; Guo et al., 2004; Tamamura et al., 2005).

Bone morphogenetic protein (BMP)

BMP was originally identified as a secreted signaling molecule that could induce endochondral bone formation. Subsequent molecular cloning studies have revealed that the BMP family consists of various molecules, including members of the growth and differentiation factor (GDF/Gdf) subfamily. BMP members have diverse biological activities during the development of various organs and tissues, as well as embryonic axis determination (Hogan, 1996). Bmp2, Bmp4, Bmp7, and Bmp14 (Gdf5) are expressed in the perichondrium and are proposed to regulate cartilage formation and joint development (Macias et al., 1997; Zou et al., 1997; Francis-West et al., 1999; Tsumaki et al., 2002). Although the unique role of each BMP during skeletogenesis is still not fully understood, inactivation of Bmp2 and Bmp4 results in death at an early stage of gestation, before the onset of chondrogenesis. Gdf5, also known as cartilage-derived morphogenic protein 1 (CDMP-1), plays a role in chondrogenesis and chondrocyte metabolism, tendon and ligament tissue formation, and postnatal bone repair (Bos et al., 2008). Gdf5 has been found to be expressed in the early interzone of articular cartilage formation and mutations of this gene are present in a number of developmental bone and cartilage diseases (Masuya et al., 2007; Bos et al., 2008). Gdf5 has at least two roles in skeletogenesis. At early stages, when it is expressed throughout the condensations, Gdf5 may stimulate recruitment and differentiation of chondrogenic cells. At later stages, Gdf5 may promote interzone cell function and joint development when its expression becomes restricted to the interzone (Storm and Kingsley, 1999; Koyama et al., 2008).

BMP antagonists

Extracellular BMP antagonists such as Noggin and Chordin can block BMP signaling by binding to BMP and preventing BMP binding to specific cell surface receptors. *Noggin* is expressed in condensing limb mesenchyme in mouse embryos, and expression persists in differentiated chondrocytes. When the *Noggin* gene was ablated, the mesenchymal condensations became much larger and limb joints failed to form, indicating that *Noggin* is critical for normal development of both long bones and joints (Brunet et al., 1998). Subsequent work in chick embryos showed that expression of *Noggin*, *Chordin*, and BMP-2 characterizes the interzone once it is established, and that *Chordin* expression persists in older developing joints, while *Noggin* expression shifts to epiphyseal chondrocytes (Francis-West et al., 1999). These and other data are widely acknowledged to signify that the action of BMP antagonists is required to regulate the pace and extent of chondrogenesis in early developing long bones, and that sustained and more restricted expression and action of these factors in developing joints would maintain the mesenchymal character of interzone cells and permit normal progression of interzone function and joint formation (Hall and Miyake, 2000). Absence of joints in *Noggin*-null mice would thus be due to exuberant and nonphysiologic action by endogenous BMPs and, consequently, rapid and abnormal conversion of the entire mesenchymal condensations into chondrocytes. Though these conclusions are plausible and quite likely, what remains unclear is how the absence of *Noggin* leads to joint ablation; specifically, whether the interzone fails to form completely or whether it starts forming but cannot be sustained, whether other BMP inhibitors fail to be activated, or whether joint formation sites fail to respond to upstream patterning cues (Pacifici et al., 2005).

GADD45ß

Growth arrest and DNA damage inducible protein 45ß (GADD45ß) has recently been found to be an important genetic regulator in endochondral ossification. GADD45ß is induced by BMP-2 via a Smad1/Runx2-dependent pathway (Ijiri et al., 2005). Due to the temporal and spatial discrepancy between Runx2 and MMP-13 expression in the development of the hypertrophic chondrocyte, Goldring et al. sought to identify an intermediate messenger connecting the expression of these molecules. They examined embryonic growth plates and found that expression of GADD45ß coincided with Runx2 protein in prehypertrophic chondrocytes, whereas GADD45ß was localized in the nucleus of late stage hypertrophic chondrocytes where MMP-13 mRNA was expressed (Goldring et al., 2006). These findings, combined with defective mineralization, decreased endochondral growth, and deficient MMP 13 and Collagen-10 expression in GADD45ß knockout mice, identified GADD45ß as a critical mediator required for MMP-13 expression during late-stage chondrocyte hypertrophy (Goldring et al., 2006). GADD45ß has also been found to be an important anti-apoptotic factor during genotoxic stress in other cell types (Goldring et al., 2008). Consistent with these findings, GADD45ß knockout

mice demonstrate enhanced TNFα-induced articular chondrocyte apoptosis (Ijiri et al., 2008). Therefore, GADD45ß is associated with down regulation of Collagen-2 activity and expression of Collagen-10 and MMP13, but it also may have important implications for prolonging survival of hypertrophic chondrocytes and preventing TNFα- induced apoptosis.

Indian hedgehog

Indian Hedgehog (Ihh) is a member of the hedgehog proteins, and is essential for skeletal development. Ihh coordinates chondrocyte proliferation, chondrocyte differentiation, and osteoblast differentiation. Ihh is synthesized by chondrocytes leaving the proliferative pool (prehypertrophic chondrocytes) and by early hypertrophic chondrocytes. Ihh knockout mice demonstrate pronounced abnormalities of chondrocyte differentiation and bone growth. All cartilage elements are small in Ihh^{-/-} mice because of a marked decrease in chondrocyte proliferation. A second skeletal abnormality of Ihh^{-/-} mice is an increase in the fraction of chondrocytes that are post-mitotic, hypertrophic chondrocytes. This abnormality results from chondrocytes leaving the pool of proliferating chondrocytes prematurely because Ihh^{-/-} cartilage fails to synthesize parathyroid hormone-related protein (PTHrP) that acts primarily to keep proliferating chondrocytes in the proliferative pool. A third striking abnormality of Ihh^{-/-} mice is the absence of osteoblasts in either the primary spongiosa or the bone collar of bones formed by endochondral development, suggesting that Ihh is required for osteoblast differentiation in endochondral bone formation (Karp et al., 2000; Kronenberg, 2003).

Ihh is a critical and possibly direct regulator of joint development. In its absence, distribution and function of GDF5 expressing interzone-associated cells are abnormal, but their patterning at prospective joint sites still occurs. In *Ihh*^{-/-} mouse embryos, cartilaginous digit anlagen remained fused and lacked interzones or mature joints (Koyama et al., 2007).

Fibroblast growth factors

Recent evidence suggests that the fibroblast growth factor (FGF) family plays an essential role in the proliferation and differentiation process of chondrocytes. The impact of many of these factors is not fully understood, but multiple FGF and FGFR (FGF receptor) genes are expressed at every stage of endochondral ossification. Within the chondrocyte pathway, FGFR3 is found in proliferating chondrocytes, FGFR1 in prehypertrophic and hypertrophic chondrocytes, and FGFR2 is expressed among the earliest condensing mesenchyme (Ornitz and Marie, 2002). FGFs markedly enhance Sox9 expression in the early stages of development, likely through the mitogen-activated protein kinase (MAPK) pathway (Murakami et al.,

2000). It is also probable that some degree of FGF-mediated signaling is related to its ability to regulate Ihh expression (Minina et al., 2002).

Vascular endothelial growth factor (VEGF)

During endochondral bone formation, invasion of blood vessels into cartilage is associated with upregulation of VEGF in hypertrophic chondrocytes and increased expression of VEGF receptors in the perichondrium. Inactivation of this factor through the systemic administration of a soluble receptor chimeric protein (Flt-(1-3)-IgG) to mice almost completely suppressed blood vessel invasion, concomitant with impaired trabecular bone formation and expansion of hypertrophic chondrocyte zone in the growth plate. Recruitment and/or differentiation of chondroclasts, which express gelatinase B/MMP-9, caused resorption of terminal chondrocytes. These findings indicate that VEGF is an essential coordinator of chondrocyte death, chondroclast function, cartilage matrix remodeling, angiogenesis and bone formation in the growth plate (Gerber et al., 1999). Upregulation of VEGF in hypertrophic chondrocytes was lacking in Runx2-deficient mice, and cartilage angiogenesis did not occur. Over-expression of Runx2 in fibroblasts induced an increase in their VEGF mRNA level and protein production by stimulating VEGF transcription. These results demonstrate that Runx2 is a necessary component of a tissue specific genetic program that upregulates VEGF during endochondral bone formation (Zelzer et al., 2001).

ERG

The ERG (Ets-related gene) transcriptional activator belongs to the *Ets* gene family of transcription factors. ERG is not only expressed at the onset of joint formation, but persists once the articular layer has developed further. A variant of ERG named C-1-1 is expressed in most epiphyseal pre-articular/articular chondrocytes in developing long bones. When C-1-1 is mis-expressed in developing chick limbs, it is able to impose a stable and immature articular-like phenotype onto the entire limb chondrocyte population, effectively blocking maturation and endochondral ossification (Iwamoto et al., 2000). More recent studies found that limb long bone anlagen of transgenic mice expressing the ERG variant C-1-1 were entirely composed of chondrocytes actively expressing collagen-9 and aggrecan as well as articular markers such as tenascin-C. Typical growth plates were absent and there was very low expression of maturation and hypertrophy markers, including Ihh, collagen-10, and Mmp13. There was a close spatio-temporal expression of both ERG and GDF5 in developing mouse embryo joints and GDF5 was an effective inducer of ERG expression. These results suggest that ERG is part of molecular

mechanisms driving the differentiation of immature chondrocytes into permanent articular chondrocytes, and may do so by acting downstream of Gdf5 (Iwamoto et al., 2007).

These previous studies suggest that mesenchymal progenitor cells differentiate into two fundamentally distinct types of cartilage cells during skeletal development. The cartilage cells in the primary and secondary ossification centers and the growth plate undergo proliferation, differentiation to functional chondrocytes, then hypertrophic differentiation, apoptosis, and eventual resorption and replacement by newly formed bone through the endochondral sequence of ossification. This type of cartilage is called temporal or replacement cartilage. In contrast, cartilage cells close to the surface of growing long bones divide and differentiate to form hyaline articular cartilage, which is termed permanent or persistent cartilage because articular chondrocytes normally do not undergo terminal differentiation or endochondral ossification and are not replaced by bone (Eames et al., 2004; Pacifici et al., 2005). The phase-specific expression and possible regulatory effects of the above-mentioned factors on chondrocyte differentiation and articular cartilage formation are summarized in Figure 1.

Regulation of adult articular chondrocyte activity and pathophysiology of OA

Mature cartilage exists in three main types: hyaline cartilage, fibrous cartilage, and elastic cartilage. Hyaline cartilage is of particular focus here because it is the innate component of those diarthrodial joint surfaces involved in OA. Articular cartilage is a smooth, glistering white tissue composed of hyaline cartilage, which is divided into four zones: the superficial tangential zone, composed of thin tangential collagen fibrils and low aggrecan content, the transitional/middle zone, composed of thick radial collagen bundles, the deep zone, composed of even thicker radial bundles of collagen fibrils, and finally the calcified cartilage zone located directly below the tidemark and superficial to the subchondral bone (Goldring and Marcu, 2009). The calcified zone persists after growth plate closure and serves as an important mechanical buffer between the uncalcified articular cartilage and the subchondral bone. Generally, cell density decreases and cell volume and relative proteoglycan content increase as the cartilage transitions from superficial to deep. Unlike those chondrocytes involved in endochondral ossification, the chondrocytes within normal articular cartilage do not
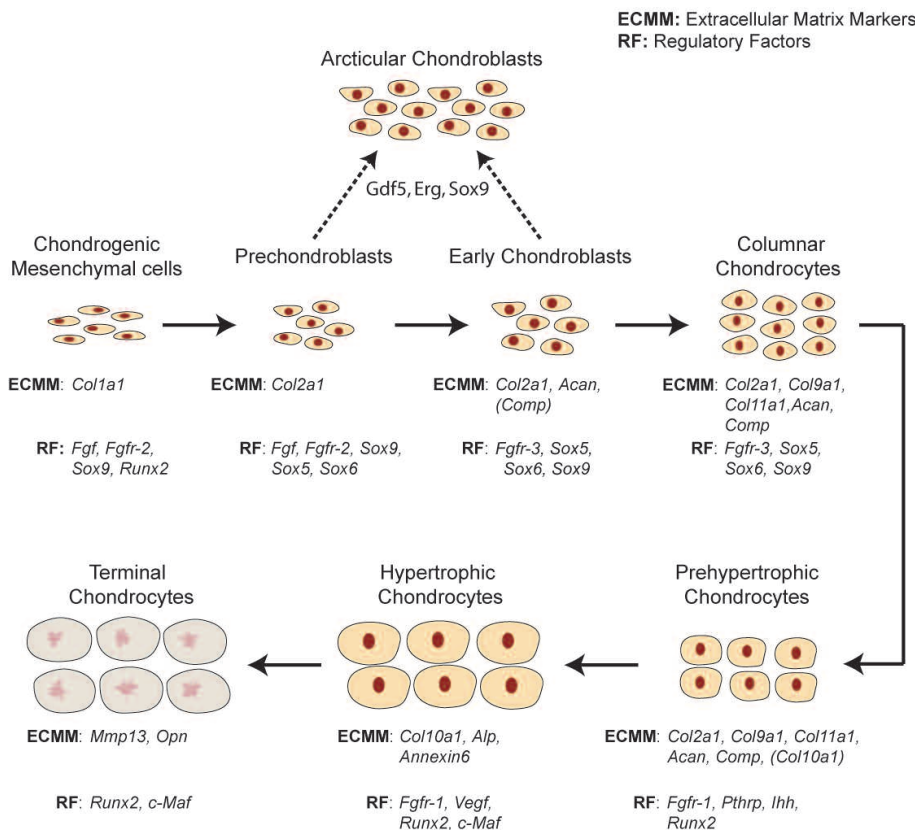

Fig. 1. A diagram shows the successive steps of chondrocyte differentiation pathways toward either articular chondrocytes or endochondral differentiation during skeletal development. Major morphological features, extracellular matrix markers, and possible regulatory factors at each step are demonstrated.

undergo terminal differentiation, but persist and function to produce extracellular matrix and maintain cartilage homeostasis.

The extracellular matrix is produced and maintained by the chondrocyte, and articular cartilage is characterized by the absence of blood vessels or nerves. Due to the avascularity of articular cartilage, some nutrients are provided by diffusion via the synovial fluid. Articular chondrocytes have adapted to the very low oxygen tension (in the range of 1-7%) and low glucose levels with facilitated glucose transport via upregulation of hypoxia inducible factor-1 (HIF-1), expression of glucose transporter-1 and -3 (GLUT1 and GLUT3), and enhanced anaerobic glycolysis (Wilkins et al., 2000; Mobasheri et al., 2005; Clouet et al., 2009). Much like bone, the physiologic properties of articular cartilage are primarily related to the extracellular matrix, but homeostasis in adult articular cartilage relies on the function of articular chondrocytes. In healthy articular cartilage, chondrocytes maintain a very low turnover rate of its constituents with a good balance of anabolism vs. catabolism.

Articular cartilage undergoes changes in its material properties related to aging which are separate from the disease process of OA, but may eventually predispose cartilage to OA or contribute to its progression. One such factor is the development of advanced glycation end products (AGEs), which enhance collagen cross-linking and make the tissue more brittle (Verzijl et al., 2002). Additionally, the ability of chondrocytes to respond to growth factor stimulation appears to decline with age, leading to decreased anabolism (Loeser and Shakoor, 2003). Chondrocytes also demonstrate increasing senescence with age due to erosion of telomere length related to oxidative stress (Martin et al., 2004). This effect is further exacerbated with exposure to repetitive mechanical loading (Martin et al., 2004).

OA is a degenerative disease process characterized radiographically by loss of articular cartilage, narrowing of joint space, subchondral bone eburnation and sclerosis, osteophyte formation, and subchondral cyst formation. In addition to bony changes evident on radiographs, OA also affects the synovium and surrounding connective tissue, indicating it is not simply a disease of articular cartilage (Brandt et al., 2006). At the cellular and molecular level, OA is a disruption of normal cartilage homeostasis, generally leading to excessive catabolism relative to anabolism. Factors that contribute to development of OA include advanced age, joint trauma, irregular joint mechanics and malaligment, obesity, muscle weakness, and genetic predisposition. Although OA is not commonly referred to as an inflammatory arthropathy due to the lack of neutrophils in the synovial fluid and the absence of a larger systemic inflammatory response, inflammatory mediators clearly play a role in the progression of OA.

In early OA, global gene expression within the chondrocyte is activated following mechanical injury causing increased expression of inflammatory mediators, cartilage-degrading proteinases, and stress-response factors (Fitzgerald et al., 2004; Kurz et al., 2005). Loss of proteoglycans and cleavage of collagen- 2 occurs initially at the surface of articular cartilage, causing reduced mechanical strength of the cartilage matrix (Goldring and Goldring, 2007). The osteoarthritic chondrocyte is activated and demonstrates the ability to divide to form clusters (Clouet et al., 2009). Chondrocytes initially attempt to synthesize and replace degraded extra-cellular matrix components such as collagen-2, -9, and -11, aggrecan, and pericellular collagen-4 (Buckwalter et al., 2007). The compensatory synthesis of ECM components is most evident among the deeper regions of articular cartilage (Fukui et al., 2008). Among the factors stimulating anabolism are insulin like growth factor-1 (IGF-1), members of the transforming growth factor ß (TGF-ß) superfamily such as BMP-2 and inhibin BA/activin, and fibroblast growth factor (Fukui et al., 2003; Hermansson et al., 2004). These attempt to offset the degradation caused by inflammatory mediators such as Interleukin-1 (IL-1), tumor necrosis factor-a (TNF-a), MMPs (such as MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14), aggrecanases (particularly the ADAMTS family such as ADAMTS-4 and ADAMTS-5) and other catabolic cytokines and chemokines.

As OA progresses, the synthetic balance shifts to favor catabolism. Significant heterogeneity in the synthetic capacity of articular chondrocytes occurs in OA cartilage. Overall gene activation is increased in the deep zone, but is decreased in the superficial zone and areas in the mid zone with degradation (Fukui et al., 2008). The chondrocyte is no longer able to create the complex matrix found in healthy articular cartilage. Evidence of phenotypic modulation of endochondral ossification, such as collagen-1, -3, -10, is found in osteoarthritic cartilage, which is not characteristic of normal adult articular cartilage (Sandell and Aigner, 2001). As radiographic imaging suggests, changes also occur in the subchondral bone that accompany articular cartilage loss. Subchondral plate thickness increases, the tidemark advances, and angiogenesis invades an otherwise avascular structure (Lane et al., 1977). Apoptosis of the chondrocyte is seen in late OA, which is mediated in part, by the caspases and inflammatory mediators such as IL-1 and Nitric Oxide (NO) (Kim and Blanco, 2007).

Osteoarthritic cartilage usually displays an imbalance between anabolic and catabolic events in favor of matrix catabolism, which leads to cartilage degradation. Another aberrant behavior of osteoarthritic chondrocytes is the presence of collagen-10 (a marker of hypertrophic chondrocytes) and other differentiation markers, including annexin VI , alkaline phosphatase (Alp), osteopontin (Opn), and osteocalcin (Pfander et al., 2001; Pullig et al., 2000a,b; von der Mark et al., 1992), indicating that OA cartilage cannot maintain the

characteristics of the permanent cartilage but adds those of the embryonic or growth plate cartilage. These studies suggest that chondrocyte maturation is likely to be deeply involved in the pathogenesis of OA. Recent findings on the regulatory effects of transcription/growth factors on the function of adult articular chondrocytes and their significance in OA are discussed below.

Sox9

As mentioned above, Sox9 is critical for chondrocyte differentiation and cartilage morphogenesis during skeletal development (Bi et al., 1999). However, the expression level and function of Sox9 in adult articular cartilage are controversial in literature. A gene expression study reported that Sox9 expression was lower in human OA cartilage (Brew et al., 2010). However, another study showed that no significant difference in subcellular expression of Sox9 protein was observed between osteoarthritic and normal control human cartilage. Sox9 overexpression does not correlate with collagen-2 expression in adult articular cartilage, suggesting that Sox9 is not the key regulator of collagen-2 expression in human adult articular chondrocytes (Aigner et al., 2003). Furthermore, in vitro studies showed that overexpression of Sox9 was unable to restore the chondrocyte phenotype of dedifferentiated osteoarthritic articular chondrocytes (Kypriotou et al., 2003). These studies suggest that although Sox9 has a crucial role in chondrocyte differentiation during skeletal development, its regulatory effect on the function of adult articular chondrocytes and pathogenesis of OA remains to be elucidated.

Runx2

The induction of Runx2, an essential transcription factor for chondrocyte hypertrophy, in articular chondrocytes under mechanical stress leads to cartilage degradation and osteophyte formation through chondrocyte maturation (indicated by expression of Collagen-10) and MMP-13 production (Takeda et al., 2001; Ueta et al., 2001; Kamekura et al., 2005, 2006). GADD45ß has been described as a probable intermediate molecule in the interaction between Runx2 and MMP-13 (Goldring et al., 2006; Ijiri et al., 2008).

Nfat1

Nfat1 is a member of the Nuclear Factor of Activated T-cells (NFAT) transcription factor family originally identified as a regulator of the expression of cytokine genes during the immune response (Hodge et al., 1996; Xanthoudakis et al., 1996). Nfat1 has recently been shown to play an important role in maintaining the permanent cartilage phenotype in adult mice. Nfat1 knockout ($Nfat1^{-/-}$) mice exhibited normal skeletal development, but displayed over-expression of numerous matrix-degrading proteinases and pro-

inflammatory cytokines and loss of collagen-2 and aggrecan at the initiation stage. These initial changes are followed by articular chondrocyte clustering, formation of periarticular chondro-osteophytes, progressive articular surface destruction, exposure of thickened subchondral bone, and formation of subchondral bone cysts (Wang et al., 2009) (Fig. 2), all of which resemble human OA (Pritzker et al., 2006). Forced expression of Nfat1 delivered with lentiviral vectors in cultured adult primary *Nfat1* knockout ($Nfat1^{-/-}$) articular chondrocytes partially or completely rescued the abnormal catabolic and anabolic activities of $Nfat1^{-/-}$ articular chondrocytes. These new findings revealed a previously unrecognized role of Nfat1 in maintaining the physiological function of differentiated adult articular chondrocytes through regulating the expression of specific matrix-degrading proteinases and pro-inflammatory cytokines (Wang et al., 2009).

Thickening of subchondral bone is one of the characteristics of human OA. Pain in the OA joint is usually secondary to synovitis and subchondral bone changes (Brandt et al., 2008). However, the precise biological mechanisms underlying the subchondral bone changes remain unclear. $Nfat1^{-/-}$ mouse joints displayed chondrocyte hypertrophy in the deep-calcified zones of articular cartilage, a feature of human OA cartilage. $Nfat1^{-/-}$ mesenchymal cells derived from subchondral bone marrow cavities differentiated into chondrocytes which subsequently underwent hypertrophy and endochondral ossification, leading to thickening of both subchondral plate and subchondral trabecular bone (Fig. 3). These findings suggest that Nfat1 may prevent the endochondral ossification pathway in adult articular cartilage and subchondral bone, thereby maintaining the integrity of cartilage-bone structure of synovial joints.

Wnt/ß-catenin signaling

The Wnt/ß-catenin signaling pathway is known to induce chondrocyte maturation and endochondral ossification during skeletal development. It is also important in osteoblast differentiation and susceptibility to OA. Recent studies revealed that the canonical Wnt signaling is also involved in chondrocyte maturation and endochondral ossification in adult chondrocytes. The inhibition of Dickkopf-1 (Dkk1), a negative regulator of the Wnt signal, has been reported to allow conversion of a mouse model of rheumatoid arthritis to OA, due to increased endochondral ossification (Diarra et al., 2007). The conditional activation of ß-catenin in articular chondrocytes of adult mice caused OA-like cartilage degradation and osteophyte formation. These pathological changes were associated with accelerated chondrocyte maturation and Mmp expressions (Zhu et al., 2009). Interestingly, the same group also reported that selective suppression of ß-catenin signaling in articular chondrocytes also caused OA-like cartilage degradation in Col2a1-ICAT (inhibitor of ß-catenin and T-cell factor) transgenic mice, and this was mediated by
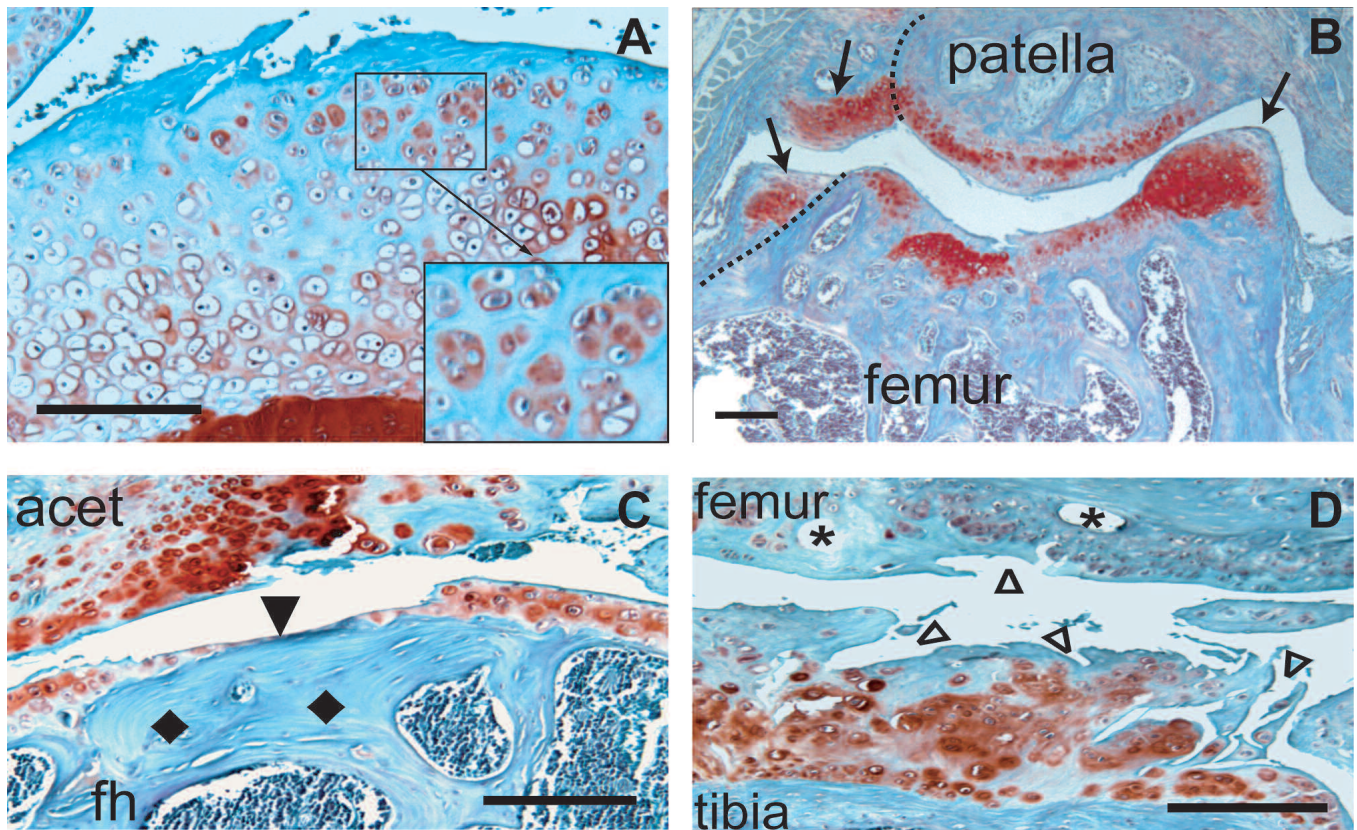
Fig. 2. Photomicrographs of Nfat1$^{-/-}$ mouse joints showing osteoarthritic changes. Safranin-O and fast green staining and counterstained with hematoxylin. Reorganized from the published figures (Wang, et al. J Pathol 2009; 219:163-72) with permission from the publisher. **A.** At 4 months of age, roughening of the articular surface, loss of safranin-O staining for proteoglycans (red), and early chondrocyte clustering (in magnified rectangle) which is a typical histologic feature of human OA are seen in the upper-mid zones of articular cartilage of an *Nfat1$^{-/-}$* femoral head. **B.** A 9-month *Nfat1$^{-/-}$* patellofemoral joint demonstrates articular cartilage degradation and the formation of chondro-osteophytes appearing as newly formed articular surfaces (arrows). Dotted lines indicate the surfaces of the original articular cartilage/bone. **C.** An 18-month *Nfat1$^{-/-}$* hip shows thinning or complete loss of articular cartilage (arrowhead) with exposure of thickened subchondral bone (black diamonds). acet, acetabulum; fh, femoral head. **D.** Articular cartilage destruction with loss of safranin-O staining, surface fibrillation (open arrowheads), and subchondral bone cysts (*) are seen in the knee joint of a 15-month-old *Nfat1$^{-/-}$* mouse. Scale bars: 200 *µm*.
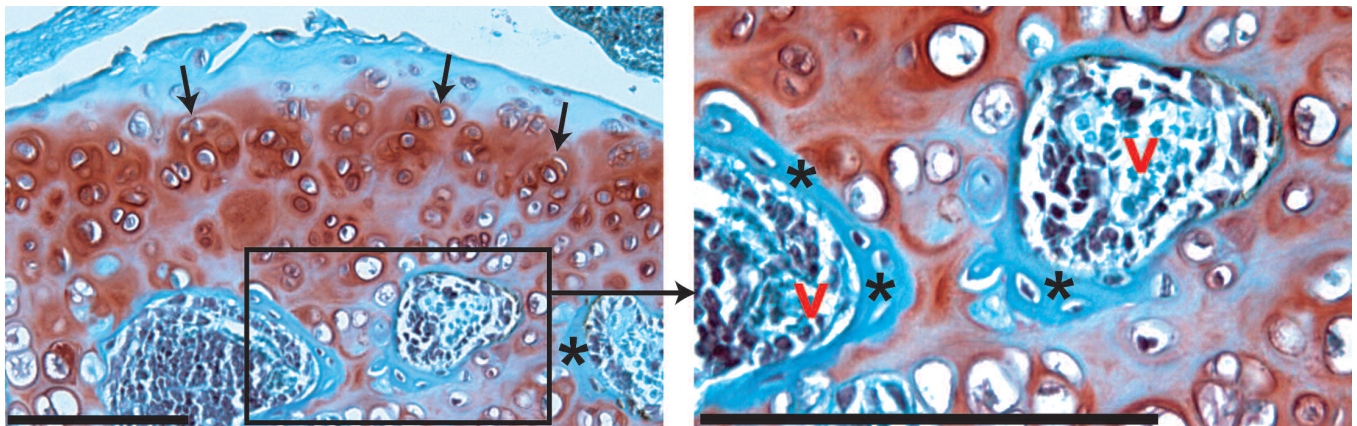

Fig. 3. A 6-month *Nfat1$^{-/-}$* hip joint shows roughening and discontinuity of articular surface, loss of safranin-O staining in the upper zone, chondrocyte clustering in the upper-mid zones (arrows), and increased safranin-O staining in mid-deep zones of femoral head articular cartilage. Endochondral ossification, including chondrocyte differentiation/hypertrophy, vascular invasion (red v), and new bone formation (*) on the surfaces of calcifying cartilage, is seen in subchondral bone (in magnified rectangle). Safranin-O and fast green staining and counterstained with hematoxylin. Modified from the published figure (Wang et al. J. Pathol. 2009; 219, 163-172) with permission from the publisher. Scale bars: 200 *µm*.

enhancement of apoptosis of the chondrocytes (Zhu et al., 2008). These results suggest that both excessive and insufficient ß-catenin levels may impair the homeostasis of articular chondrocytes by enhancing pathological maturation and apoptosis, respectively, both of which are endochondral ossification processes.

GDF-5

Multiple polymorphisms of the GDF-5 gene have been found to produce an increased Odds Ratio of OA development, but the one that appears to have the most robust correlation is the rs143383 SNP in the 5'-UTR of GDF5. Miyamoto et al. reported a strong association between the rs143383 SNP of GDF5 and hip and knee OA in multiple Asian populations, with Odds Ratios ranging from 1.30-1.79 (Miyamoto et al., 2007). This association was confirmed in a large-scale meta-analysis; however, the magnitude of effect was less than

previously reported (Evangelou et al., 2009). Interestingly, Egli et al. found that GDF5 expression imbalance was not limited to articular cartilage, but found in multiple joint tissues analyzed (synovium, fat pad, meniscus, ligaments) (Egli et al., 2009).

Figure 4 present factors that may be responsible for the maintenance of the physiological function of adult articular chondrocytes and development of OA.

Current therapies for OA

As mentioned earlier, a multitude of therapeutic interventions currently exist for the symptomatic control of OA. It is important to note though, that none of the current therapies can cure OA. The treatment options include total joint arthroplasty which has been employed for many decades, as well as autologous chondrocyte transplantation which has evolved more recently. Even more will become available in the near future as
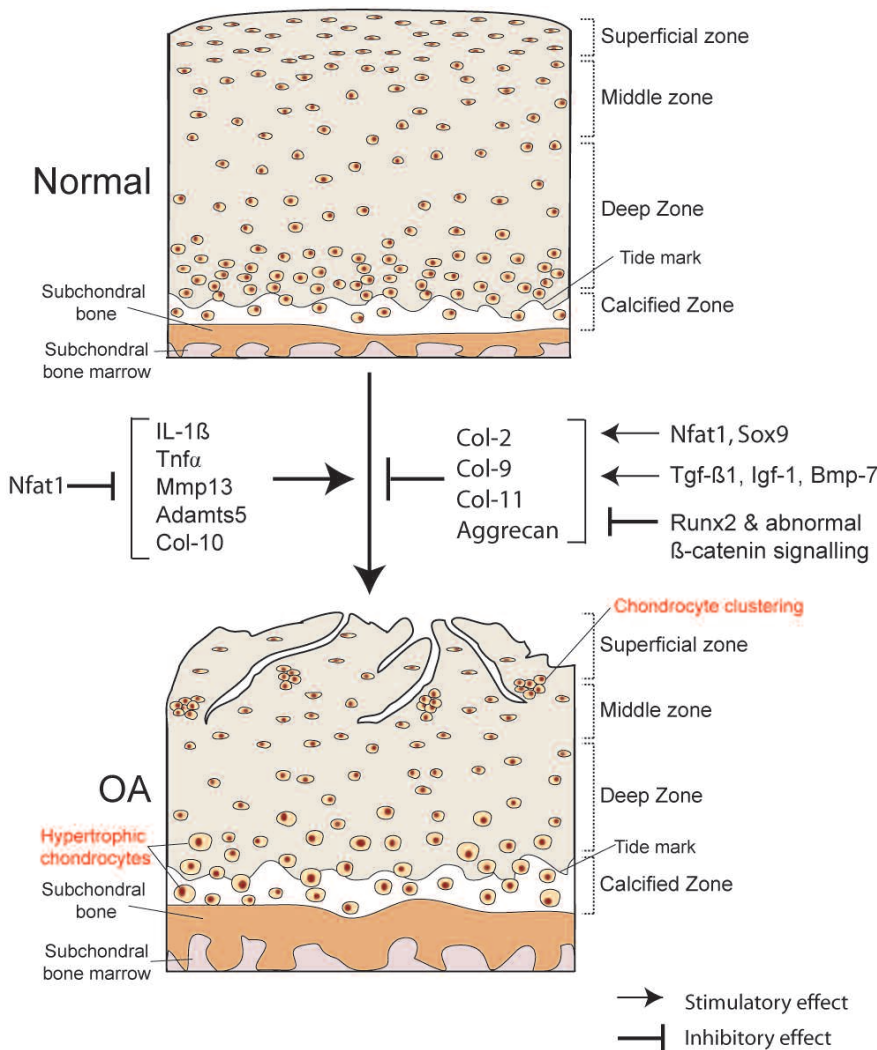

Fig. 4. A diagram demonstrates factors that may be responsible for the maintenance of the physiological function of adult articular chondrocytes and the development of OA.

understanding of the molecular framework intrinsic in the development of OA grows, and its clinical application is refined. The current options can be categorized generally as (1) pharmaceutical or (2) surgical intervention.

Pharmaceutical management

Pharmaceutical management may be effective in mild to moderate OA. Acetaminophen is the most commonly used drug in the treatment of OA due to its analgesic effects (Hochberg et al., 1995). Although its mechanism of action is not fully understood, it likely exerts its action by modifying the cyclooxygenase system without affecting the inflammatory cascade. Although it has no known disease-modifying properties, its excellent safety profile at appropriate doses makes it a commonly used first-line medication. Another frequently used non-narcotic analgesic is Tramadol. Tramadol can be used synergistically with Acetaminophen for improved pain relief, and like Acetaminophen, Tramadol has no known disease-modifying or anti-inflammatory properties.

NSAIDs are a large group of pharmaceuticals that have both analgesic and anti-inflammatory effects. There is significant individual variation in the pharmacologic properties of these drugs, but in general, they target the COX-1 and COX-2 isoenzymes responsible for generating prostaglandins, a major inflammatory mediator. The COX-2 isoenzyme is an inducible form exhibited only in activated inflammatory cells and more recent NSAIDs have been utilized to selectively inhibit this protein. However, due to concerns of cardiovascular toxicity and the possible increased risk of a cardiac event, the routine use of these medications has been the subject of scrutiny. Overall, NSAIDs are effective medications for the management of mild-moderate OA, though the side-effect profile, including possible gastrointestinal, renal, and cardiovascular toxicity remains their largest limitation.

Intra-articular injection therapy also plays a role in the clinical management of OA, particularly in joints that are easily accessible such as the knee, shoulder, ankle, and metacarpal joints. Currently approved therapies fall into one of two categories, corticosteroid-based or hyaluronic acid derivatives. Corticosteroids have been used for many years and remain a reasonable and cost-effective method of short-term pain control due to their potent anti-inflammatory capabilities (Bellamy et al., 2006). These drugs have not been shown to prevent or slow the natural progression of OA though, and if used too frequently, may have deleterious effects on cartilage and joint structure (Raynauld et al., 2003; Behrens et al., 1975). Hyaluronic acid is a poly-saccharide synthesized and secreted by type B synoviocytes found naturally in the synovial fluid of diarthrodial joints (Buckwalter et al., 2007). Hyaluronic acid contributes many important visco-elastic properties to the function of articular cartilage, including lubrication, chondrocyte protection during loading, and molecular and cellular communication (Buckwalter et al., 2007). The use of intra-articular injections of hyaluronic acid for OA treatment is termed "viscosupplementation." Multiple low and high molecular weight preparations of hyaluronic acid are Food and Drug Administration (FDA)-approved for OA treatment, and they have been found to increase synthesis of glycosaminoglycan and collagen-2 *in vitro* via the CD44 cell surface receptor (Akmal et al., 2005).

Anti-inflammatory effects of hyaluronan have also been reported, such as the suppression of IL-1, induced metalloproteinase (Waddell et al., 2007). Generally though, the disease-modifying chondroprotective effects of hyaluronic acid have not been well proven. Several randomized trials and meta-analyses have demonstrated improved function and pain relief with hyaluronic acid when compared to placebo and corticosteroids, validating its position as an effective therapy for symptomatic control of OA (Lo et al., 2003; Arrich et al., 2005). The most recent clinical practice guideline on the treatment of OA of the knee recommends intra-articular corticosteroids for short-term pain relief, but cannot recommend for or against the use of intra-articular hyaluronic acid for patients with symptomatic OA of the knee (Richmond et al., 2010).

Dietary supplementation with chondroitin and glucosamine, as well as other vitamins and minerals linked to the development of OA, has gained increasing attention in recent years. The evidence thus far to support efficacy in symptomatic control and slowing of radiographic disease has been conflicting. A meta-analysis found highly statistically significant effects on both symptom control and slowing of radiographic disease (Richy et al., 2003). A later meta-analysis, however, was much less conclusive and found limited benefit of glucosamine over placebo in pain control and functional improvement (Towheed et al., 2005). Both glucosamine and chondroitin have demonstrated excellent safety profiles and have limited potential to harm the patient, but further studies are required before universal endorsement of their application can be encouraged. The most recent clinical practice guideline on the treatment of OA of the knee recommends glucosamine and/or chondritin not be prescribed for patients with symptomatic OA of the knee (Richmond et al., 2010). Dietary deficiencies of Vitamin K, Vitamin D, and Selenium have been found to increase the risk of OA development. However, the few studies performed to date have not shown benefit from routine supplementation (Neogi et al., 2006; Felson et al., 2007).

Surgical intervention

Surgical intervention for OA spans a broad continuum of options ranging from minimally invasive arthroscopic procedures, extra-articular realignment procedures, and partial or total joint arthroplasty. Surgical options are used in conjunction with

pharmacologic therapy, physical therapy, and are tailored to each patient based on factors including severity of disease, radiographic findings, level of functioning, and success or lack thereof, of previous therapeutic interventions.

Arthroscopic procedures are typically reserved for patients with an intra-articular injury, such as a meniscal tear, or those with focal osteochondral pathology. These individuals may benefit from procedures such as microfracture, Pridie drilling or abrasion chondroplasty, which aim to stimulate a spontaneous repair response by creating a conduit to the subchondral bone spaces, allowing mesenchymal stem cells and other molecular mediators to create a blood clot at the site of chondral injury (Hunziker, 2002; Frisbie et al., 2003). Analysis of the repair tissue has found that repair cartilage is predominantly fibrocartilage in nature, yielding less beneficial mechanical properties than the innate hyaline cartilage (Clouet et al., 2009). Clinical outcomes have been reasonably good with this procedure, particularly in younger patients, but for older patients with a more limited capacity for repair or those with more diffuse disease, its efficacy is less significant (Steadman et al., 2003; Miller et al., 2004; Yen et al., 2008). Arthroscopic lavage and debridement have been shown to be no better than a placebo. Arthroscopic procedures for the treatment of OA should be limited to those with mechanical symptoms, mild to moderate arthritis, or imaging that demonstrates limited, focal chondral pathology (Siparsky et al., 2007; Dearing and Nutton, 2008; Richmond, 2008).

Autologous chondrocyte implantation (ACI) was first reported by Brittberg et al. in 1994 and has been met with growing popularity in the treatment of full-thickness articular cartilage lesions (Brittberg et al., 1994). ACI is a staged procedure that involves arthroscopically harvesting chondrocytes from a non- or less-weight-bearing area of the medial trochlear groove of the knee, culturing these cells in vitro, and implanting the chondrocytes in the target lesion during a second arthroscopic procedure. First generation ACI utilized an autologous periosteal flap to cover the implanted chondrocytes and seal them within the lesion. However, periosteal flap hypertrophy was commonly encountered at follow-up and newer procedures have replaced the periosteal flap with collagen or hyaluronic acid-based membranes to limit this effect. Much like microfracture, concern exists regarding the quality of the repair tissue that is generated after ACI. Although clinical reports are often good, histologic analysis of short-term results indicated that repair tissue was often fibrocartilage in nature. However, Gikas et al. recently demonstrated that the implanted chondrocytes undergo prolonged multiphase remodeling and are significantly more likely to have hyaline-like characteristics if biopsied at a later time, such as two years post-implantation (Gikas et al., 2009). ACI is currently indicated for the treatment of focal chondral defects.

Mechanical realignment procedures such as the high

tibial osteotomy have been performed for many decades and remain a reasonable treatment method for the appropriate patient with unicompartment arthrosis. This has been studied and performed most extensively with a varus deformity of the knee, medial compartment arthrosis, and subsequent valgus-producing tibial osteotomies. A recent meta-analysis again supported the improvement in pain and function that can be expected with this procedure in the appropriate patient (Brouwer et al., 2005). A distal femoral osteotomy can be performed for lateral compartment arthrosis with similar improvement to the high tibial osteotomy, but because of technical difficulty and the patient group that typically displays this form of arthritis, it is less frequently utilized (Richmond, 2008; Backstein et al., 2007).

For the patient that has failed conservative therapy or previous non-arthroplasty surgery, or is not a candidate for arthroscopic or osteotomy surgery, partial or total joint replacement is the remaining option. Unicompartmental arthroplasty can be utilized as a cost-effective method of treatment in lower demand patients than those considered for osteotomy, or older patients with unicompartmental arthrosis who may be able to avoid an eventual total joint arthroplasty (Slover et al., 2006; Soohoo et al., 2006). Unicompartmental arthroplasty is an effective method for treating isolated medial or lateral compartment knee arthritis with a normal mechanical axis, and can delay the need for total knee arthroplasty for 10 or more years. The most successful surgical intervention for OA, however, continues to be total joint arthroplasty. More than 300,000 total knee arthroplasties are performed each year within the United States Medicare population alone, and that number is expected to increase significantly in the future (Richmond, 2008). Both hip and knee arthroplasty significantly improve quality-adjusted life years, and a recent study found total joint arthroplasty to be more cost-effective than coronary artery bypass surgery (Rasanen et al., 2005). Despite its clear and well-documented results though, total joint arthroplasty has a number of drawbacks. First, as the name implies, this involves the complete or near complete removal of the articular cartilage surfaces within a joint. The articular surface is replaced with synthetic materials with a finite lifespan, necessitating a revision surgery if the patient outlives the functional lifespan of the implants. Second, primary joint arthroplasty is not a benign procedure and although risks of complications such as infection, deep vein thrombosis, and pulmonary embolus are relatively low, they can have catastrophic consequences when encountered. Furthermore, the risk profile of surgery increases with subsequent revision surgery. Finally, arthroplasty will not return patients to a pre-arthritic level of function because there are no implants that can replicate the biomechanical characteristics of the native joint. For these reasons, one can appreciate the importance of understanding the molecular events that lead to the development of OA, and identifying potential targets to slow or halt disease

progression.

Future Perspectives

The recent abundance of cellular and molecular data involving the progression of OA offers a growing number of possibilities for intervention. The options for surgical intervention will expand as the field of tissue engineering and the use of surgical biologics continues to mature. Currently applied techniques such as autologous osteochondral grafting (Mosaicplasty) and ACI have experienced limited success because they do not consistently produce hyaline-like repair cartilage tissue. Tissue engineering aims to create a repair tissue identical to the native tissue that was degraded. Cartilage tissue engineering consists of three basic constituents, including a matrix scaffold, chondrocytes or chondrogenic cells, and signaling molecules that mediate the proliferation and differentiation of cartilage cells in repair tissue. This tissue can then be grown entirely in vitro prior to implantation, or implanted after a short term of in vitro growth and allowed to mature in vivo (Hunziker, 2002; Payne et al., 2010). The tissue generated can be implanted arthroscopically, limiting the iatrogenic injury of an extensive open procedure. Theoretically this approach will also overcome the limitations of current therapies such as escape of cells from the site of repair and lack of anabolic cell mediators necessary for growth. Gene therapy is another approach to cartilage repair, which could provide a means of locally introducing molecular mediators necessary to modulate the repair of articular cartilage (Evans et al., 2009). However, these developing therapeutic interventions are primarily reserved for an intra-articular injury or focal osteochondral lesions. The development of effective therapies for extensive osteoarthritic changes would require a more sophisticated understanding of the pathogenesis of OA and comprehensive interventions.

Over the past two decades, clinical trials applying a proinflammatory cytokine or proteinase inhibitor as a candidate disease-modifying OA drug (DMOAD) have been unsuccessful due to insufficient efficacy and/or severe side effects. A large number of candidate DMOADs have been tested but none have been approved by American or European drug regulatory agencies (Hellio Le Graverand-Gastineau, 2009; Kawaguchi, 2009); suggesting that inhibition of a single catabolic molecule may not be sufficient for the treatment of OA because multiple catabolic factors are involved in its pathogenesis. Since specific transcriptional signaling molecules (e.g. Nfat1, Runx2, ß-catenin) affect the expression of multiple catabolic and/or anabolic factors in articular chondrocytes, these regulatory factors may play more important roles in the development of OA than a single catabolic proteinase/cytokine. Furthermore, OA not only affects articular cartilage but also involves other joint tissues such as the subchondral bone, synovium, capsule, menisci, and ligaments. Pathological changes in these joint tissues may affect the biological and mechanical properties of articular cartilage. Excessive repetitive loading of articular surfaces due to posttraumatic joint incongruity, instability and malalignment, obesity, or joint dysplasia may lead to joint degeneration (Buckwalter and Lane, 1997). However, contradictory findings have been reported in different types of mouse models of OA and in clinical studies (Lapvetelainen et al., 2001, 2002). Changes in morphology or biomechanical properties of the articular cartilage during aging may alter the physicochemical stimuli and the metabolism of chondrocytes during loading (Jin et al., 2001; Liu et al., 2001). Mechanical stress may also affect the release of OA biomarkers (O'Kane et al., 2006; Piscoya et al., 2005). Thus, insufficient recognition of pathological changes in other joint tissues and mechanical influence on the pathogenesis of OA may also negatively affect the efficacy of DMOAD candidates (Brandt et al., 2008). These recent research advances in the pathogenesis of OA may lead to the development of novel and effective therapeutic strategies using more up-stream pharmaceutical targets such as transcriptional signaling molecules, combined with biomechanical correction of abnormal joint loading if necessary, for the prevention and treatment of human OA.

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