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Transcriptional Regulation of Articular Chondrocyte Function and Its Implication in Osteoarthritis

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1. Introduction

Osteoarthritis (OA) is characterized by joint pain and stiffness with radiographic evidence of joint space narrowing, osteophytes, and subchondral bone sclerosis. Current treatments primarily target symptomatic control of OA, including pharmacologic therapy, local joint injection, and surgical interventions. Pharmaceuticals such as nonsteroidal anti-inflammatory drugs (NSAIDs) and Acetaminophen are aimed 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 delay the need for joint replacement. However, all these therapies are aimed at symptomatic control and have limited impact on impeding or reversing the progression to advanced OA. Therefore, interest has been high in development of structure or disease-modifying OA drugs (DMOADs) aimed at slowing, halting, or reversing the progression of structural damage of articular cartilage. A large number of candidate DMOADs have been tested but none have been approved by American or European regulatory agencies (Hellio Le Graverand-Gastineau, 2009; Lotz & Kraus, 2010).

A critical barrier in drug development for OA is that molecular and cellular mechanisms for the development of OA, especially the mechanisms that control the activity of adult articular chondrocytes, remain unclear. Overexpression of proinflammatory cytokines such as Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Goldring, 2001; Fernandes et al., 2002), matrix-degrading proteinases such as matrix metalloproteinases (MMPs) (Burrage et al., 2006) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) (Glasson et al., 2005; Karsenty, 2005; Stanton et al., 2005), and nitric oxide (Pelletier et al., 2000; Haudenschild et al., 2008; Lotz, 1999) may cause cartilage degradation. However, no single cytokine or proteinase can stimulate all the metabolic reactions observed in OA. Due to the involvement of multiple proteinases and proinflammatory cytokines in the pathogenesis of OA, a candidate DMOAD that inhibits a single proteinase or inflammatory

cytokine is unlikely to produce long-term benefit if other catabolic factors are not blocked. Therefore, it is critical to identify which upstream factors regulate the expression of these catabolic molecules in articular cartilage and other joint tissues (e.g., synovium).

This chapter summarizes the recent advances in identification of potential upstream regulators such as transcription factors and specific growth factors that may control the expression of anabolic and/or catabolic molecules in articular cartilage and their functional implications in the pathogenesis and treatment of OA.

2. Dysfunction of adult articular chondrocytes and its significance in OA

It has been recognized that OA may develop as a result of: 1) abnormal loading on normal joint tissues (articular cartilage and subchondral bone); 2) normal/physiologic loading on abnormal/defective joint tissues; or 3) a combination of the two (Piscoya et al., 2005; Brandt et al., 2008; Segal et al., 2009; Buckwalter et al., 2004; Block & Shakoor, 2009; Drewniak et al., 2009; June & Fyhrie, 2008; Borrelli et al., 2009; van der Meulen & Huiskes, 2002; Rothschild & Panza, 2007). Dysfunction of articular chondrocytes may be the initial change in OA with normal loading on abnormal articular cartilage. For OA associated with abnormal loading on normal joint tissues, dysfunction of articular chondrocytes is not the initial cause. However, mechanical disruption of the extracellular matrix and abnormal mechanotransduction in articular chondrocytes during and after joint injury or malalignment may activate specific signaling pathways, leading to changes in gene expression and cartilage metabolism as a result of dysfunction of articular chondrocytes. In order to develop effective strategies for prevention and treatment of OA, it is critical to understand the regulatory mechanisms of articular chondrocyte function.

Mature cartilage exists in three main types: hyaline cartilage, fibrocartilage, and elastic cartilage. Hyaline cartilage is characterized by matrix containing type-II collagen (collagen-2) fibers, glycosaminoglycans, proteoglycans, and multiadhesive glycoproteins. Fibrocartilage is characterized by abundant type-I collagen (collagen-1) fibers in addition to the matrix material of hyaline cartilage. Elastic cartilage is characterized by elastic fibers and elastic lamellae in addition to the matrix material of hyaline cartilage. Elastic cartilage is found in the external ear, the wall of the external acoustic meatus, the eustacchium, and the epiglottis of the larynx (Ross & Pawlina, 2006). Hyaline cartilage is of particular focus here because it is the innate component of diarthrodial joint surface involved in OA. Normal articular cartilage is composed of hyaline cartilage, which is divided into four zones: 1) the superficial tangential zone, composed of thin tangential collagen fibrils and low aggrecan content, 2) the middle or transitional zone, composed of thick radial collagen bundles, 3) the deep zone, composed of even thicker radial bundles of collagen fibrils, and 4) the calcified cartilage zone located between the tidemark and the subchondral bone (Goldring & 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 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. 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 of 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 different 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 & Shakoor, 2003). Chondrocytes also demonstrate increasing senescence with age due to erosion of telomere length related to oxidative stress.

OA is a disease process characterized radiographically by narrowing of joint space due to loss of articular cartilage, subchondral bone 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; Brandt et al., 2008). At the cellular and molecular level, OA is a disruption of normal cartilage homeostasis, generally leading to excessive catabolism relative to anabolism. Risk factors that contribute to development of OA include advanced age, joint trauma, irregular joint mechanics and malalignment, 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 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 or biological abnormalities 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 an increase in water content and reduced tensile strength of the matrix (Goldring & Goldring, 2007). Chondrocyte clustering is one of the typical features of OA cartilage (Clouet et al., 2009). Chondrocytes initially attempt to synthesize and replace degraded extracellular matrix (ECM) components such as collagen-2, -9, and -11, aggrecan, and pericellular collagen-4 (Buckwalter et al., 2007). This 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-β (TGF-β) superfamily, and fibroblast growth factors (FGFs) (Fukui et al., 2003; Hermansson et al., 2004). These attempt to offset the degradation caused by inflammatory mediators such as IL-1β, TNF-a, MMPs (e.g., MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14), aggrecanases (e.g., ADAMTS-4 and ADAMTS-5), and other catabolic cytokines and chemokines.

As OA progresses, the synthetic balance shifts to favor catabolism, which leads to cartilage degradation. 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). 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 & Aigner, 2001). OA changes also occur in the subchondral bone that accompanies 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 OA cartilage, which is mediated in part, by the caspases and inflammatory mediators such as IL-1 and Nitric Oxide (NO) (Kim & Blanco, 2007).

Another indication of 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; Pullig et al., 2000b; 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 observations 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 the pathogenesis of OA are discussed below.

3. Regulation of articular chondrocyte function and its implication in pathogenesis of OA

Many governing factors that are critical for skeletal development have also been found to have a significant involvement in adult chondrocyte homeostasis and pathophysiology of OA. For instance, during skeletal development, chondrocytes in the growth plate undergo hypertrophic and apoptotic changes and cartilage is degraded and replaced by bone. One of the current hypotheses is that OA reflects the inappropriate recurrence of the hypertrophic pathway in articular chondrocytes. To better understand the regulatory mechanisms of various transcription and non-transcription factors in articular chondrocyte function, it is necessary to include their regulatory roles in chondrocyte differentiation, endochondral ossification, and joint formation during skeletal development here in this chapter.

3.1 Chondrocyte differentiation and joint formation during skeletal development

3.1.1 Chondrocyte differentiation and endochondral ossification

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. Limb development first begins with the formation of mesenchymal condensations and the subsequent formation of a surrounding cartilaginous envelope called the perichondrium. The progenitor cells proceed to form the anlagen, or cartilaginous template of each bone. Cartilage cells undergo proliferation, differentiation to functional chondrocytes, hypertrophic differentiation, apoptosis, calcification of cartilage, 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.

3.1.2 Joint formation

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 cytodifferentiation 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 (Pacifici et al., 2005; Mitrovic, 1978).

3.1.3 Bone morphogenetic proteins (BMPs) and their antagonists in joint formation

BMPs are members of the TGF-β superfamily. BMP was originally identified as a secreted signaling molecule that could induce chondrocyte differentiation and 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; Sampath et al., 1990; Wozney et al., 1988). BMP2, BMP4, BMP7, and BMP14 (GDF5/Gdf5) are expressed in the perichondrium and are proposed to regulate cartilage formation and joint development (Francis-West et al., 1999; Macias et al., 1997; Zou et al., 1997; Tsumaki et al., 2002). Gdf5, also known as cartilage-derived morphogenetic 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). 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, Gdf5 may stimulate recruitment and differentiation of chondrogenic cells when it is expressed throughout the condensations. At later stages, Gdf5 may promote interzone cell function and joint development when its expression becomes restricted to the interzone (Storm & Kingsley, 1999; Koyama et al., 2008).

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 & 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).

3.1.4 ERG regulates the differentiation of immature chondrocytes into permanent articular chondrocytes

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 relationship in the expression of both ERG and GDF5 that is an effective inducer of ERG expression in developing mouse embryo joints. These results suggest that ERG is part of the 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 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, 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).

3.2 Transcriptional regulation of articular chondrocyte function

Factors that have been reported to regulate chondrocyte differentiation during development and articular chondrocyte function in the adult stage are discussed below.

3.2.1 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). Sox9 is expressed predominantly by mesenchymal progenitor cells and proliferating chondrocytes, but is not found in hypertrophic chondrocytes or osteoblasts (Zou et al., 2006). 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, Sox5 and Sox6 may also be essential for cartilage formation (Smits et al., 2001). Sox9 up-regulates the expression of chondrocyte-specific marker genes encoding collagen-2, collagen-9, collagen-11, and aggrecan by binding to their enhancer sequences (Evangelou et al., 2009). Sox9 also acts cooperatively with Sox5 and 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). It has been 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 morphogenetic protein-2 (BMP-2), and fibroblast growth factor-2 (FGF-2) on developing chondrocytes also appear to be mediated, in part, by Sox9 (Leung et al., 1998; Lefebvre et al., 1998; Goldring et al., 2008; Kolettas et al., 2001; Zehentner et al., 1999).

Despite the fact that Sox9 is critical for chondrocyte differentiation and cartilage morphogenesis during skeletal development (Bi et al., 1999), the expression 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 no significant difference in subcellular expression of Sox9 protein between osteoarthritic and normal control human cartilage. Sox9 overexpression did not correlate with collagen-2 expression in adult articular cartilage, suggesting that Sox9 is not a 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 plays a crucial role in chondrocyte differentiation during skeletal development, its regulatory effect on the function of adult articular chondrocytes and development of OA remains to be elucidated.

3.2.2 Runx2

The transcription factor Runx2 (also called Cbfa1, Osf2, or AML3) is a member of the Runt family of transcription factors. Runx2 has been identified as a master regulator of osteoblast differentiation. 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., 1999; Coffman, 2003; Takeda et al., 2001; Ducy et al., 1997; Komori et al., 1997; Otto et al., 1997). Histologic analyses of Runx2$^{-/-}$ mice have revealed delayed maturation of chondrocytes, indicating that Runx2 is involved in both osteogenesis and chondrogenesis (Inada et al., 1999). Eames et al. 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). Among the chondrogenic phenotypes during endochondral ossification, Runx2 is expressed mainly in the prehypertrophic and, to a lesser extent, in the late hypertrophic chondrocytes (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). Growth arrest and DNA damage induceable-45β (GADD45β) has been described as a probable intermediate molecule in the interaction between Runx2 and MMP-13 (Goldring et al., 2006; Ijiri et al., 2008; Ijiri et al., 2005).

In adult mice, Runx2 is expressed in the articular cartilage of wild-type mice at the early stage of post-traumatic knee OA, induced by surgical transection of the medial collateral ligament and resection of the medial meniscus. In this mouse model of OA, Runx2 expression in osteoarthritic cartilage parallels collagen-10 expression but arises earlier than Mmp13. After induction of post-traumatic knee OA by the same surgical procedure, $Runx2^{+/-}$ mice displayed decreased cartilage destruction and osteophyte formation, along with reduced expression of collagen-10 and MMP13, as compared with wild-type mice (Kamekura et al., 2006). Human OA cartilage exhibits increased Runx2 expression when compared to control cartilage. Runx2 co-localizes with MMP13 in chondrocyte clusters of OA cartilage. Runx2 overexpression in cultured chondrocytes increases MMP 13 expression (Wang et al., 2004).

These findings suggest that Runx2 regulates chondrocyte differentiation both in the developmental and adult stages. Runx2 may stimulate the progression of OA by promoting articular chondrocyte hypertrophy (indicated by expression of Collagen-10) and expression of MMP13 in articular cartilage (**Figure 1**).

3.2.3 Nfat1

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). Recent studies have shown that Nfat1 plays an important role in maintaining the permanent cartilage phenotype in adult mice. Nfat1 knockout (*Nfat1*$^{-/-}$) mice exhibited normal skeletal development, but displayed most of the features of human OA in load-bearing joints in adults (Wang et al., 2009; Rodova et al., 2011). *Nfat1*$^{-/-}$ articular cartilage shows overexpression of multiple specific proinflammatory cytokines (**Figure 2A-B**) and matrix-degrading proteinases (**Figure 3A**) at the initiation stage of OA (2-4 months of age). These initial changes are followed by articular chondrocyte clustering, formation of chondro-osteophytes, progressive articular surface destruction, formation of subchondral bone cysts, and exposure of thickened subchondral bone (Wang et al., 2009), all of which resemble human OA (Pritzker et al., 2006). The expression of mRNA for cartilage structural proteins (e.g., collagen-2, -9, and -11) was down-regulated but mRNA for collagen-10 was up-regulated at 2-4 months of age (**Figure 3B**). However, both collagen-2 and collagen-10 were up-regulated to varying degrees at 6 and 12 months, suggesting that early dysfunction of articular chondrocytes triggers repair activity within the degenerating cartilage (Rodova et al., 2011). These new findings revealed a previously unrecognized role of Nfat1 in maintaining the physiological function of differentiated adult articular chondrocytes by regulating the expression of specific matrix-degrading proteinases and proinflammatory cytokines.

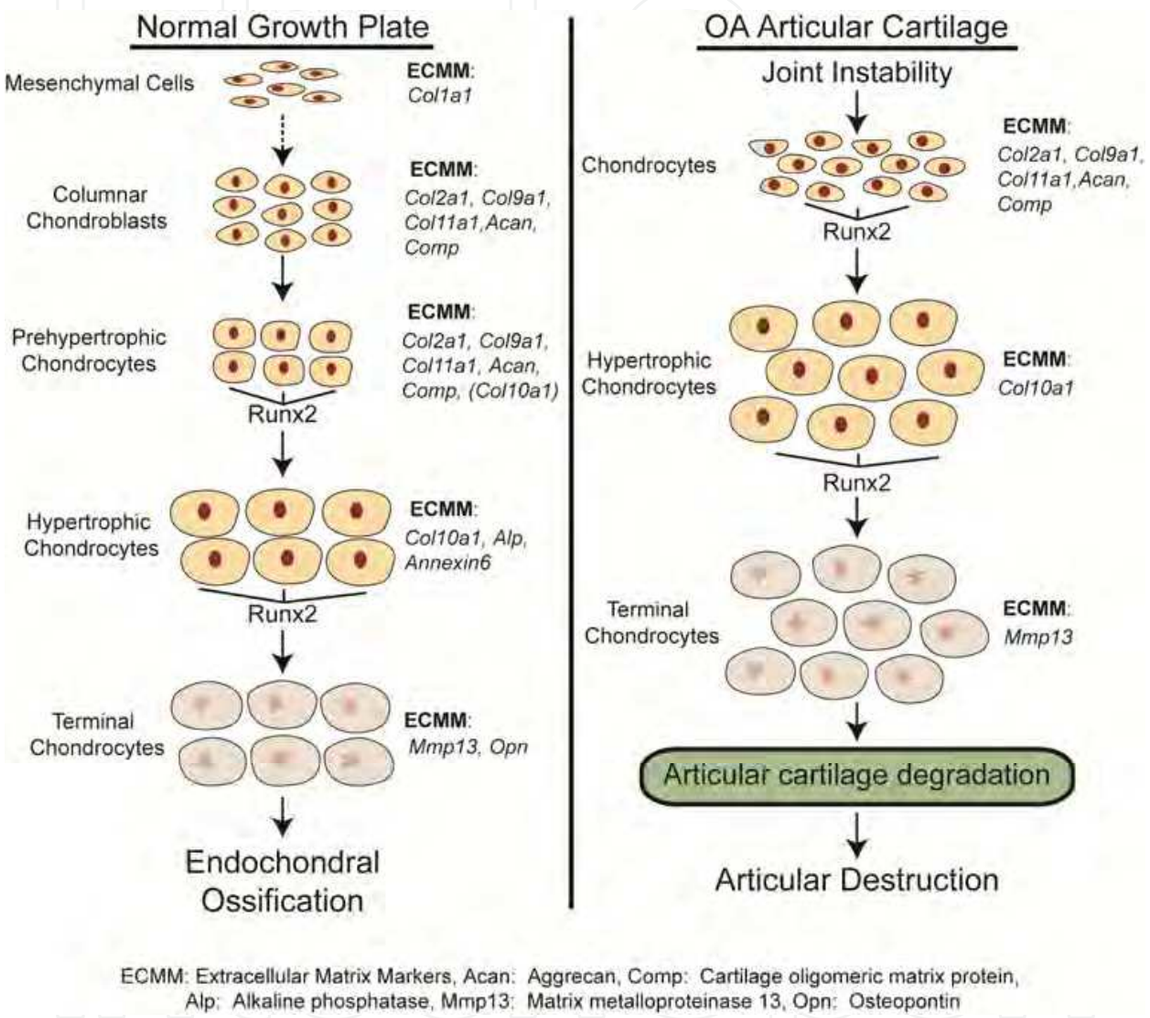

Fig. 1. A diagram shows Runx2 involvement in the chondrocyte differentiation pathways toward either physiological endochondral ossification in the growth plate or pathological chondrocyte hypertrophy in OA cartilage. Major morphological features and extracellular matrix markers for each step of chondrocyte differentiation are indicated.

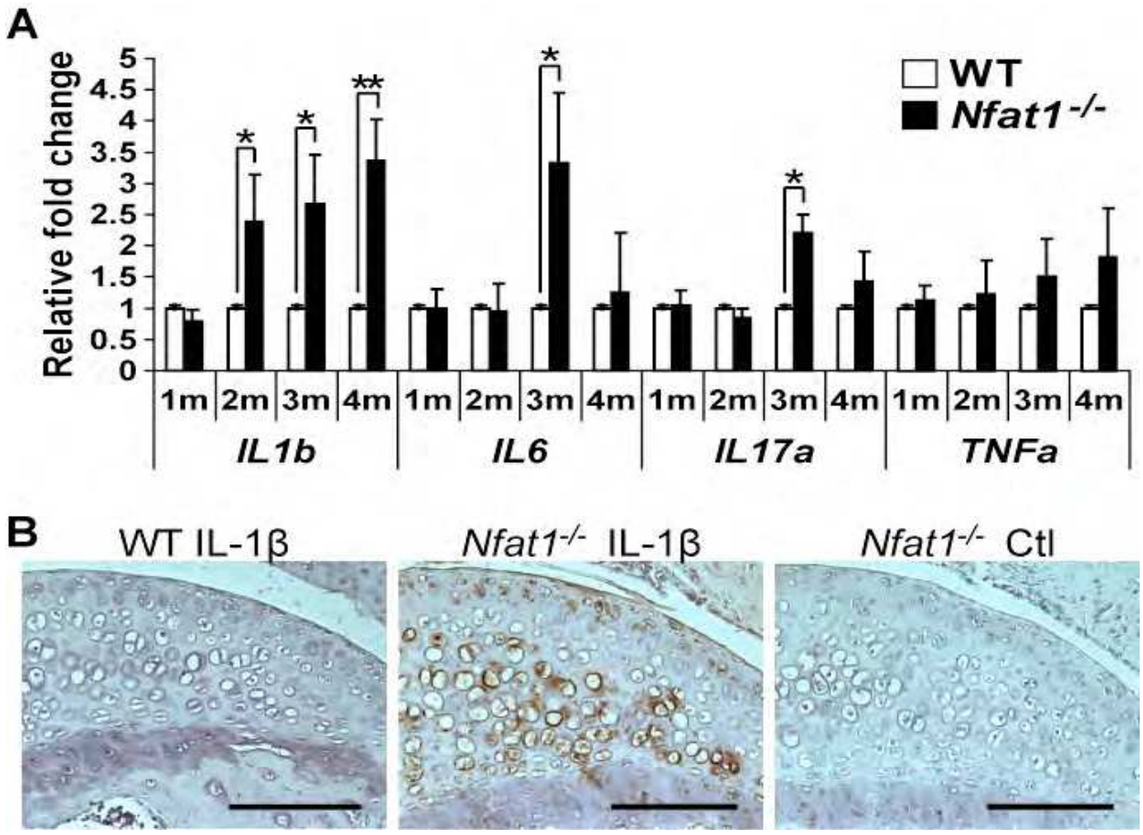

Fig. 2. Nfat1 deficiency causes dysfunction of adult articular chondrocytes with overexpression of specific proinflammatory cytokines. (**A**) Quantitative real-time PCR (qPCR) analyses indicate temporal changes in expression levels of various genes of proinflammatory cytokines in *Nfat1-/-* articular cartilage at 1-4 months (1-4m) of age. The expression level of each WT group has been normalized to "one". n = 3 pooled RNA samples, each prepared from the articular cartilage of 6-8 femoral heads. * $P < 0.05$; ** $P < 0.01$. (**B**) Immunohisto- chemical analyses using a polyclonal antibody against IL-1β (Santa Cruz) show substantially more intense expression of IL-1β (brown areas) in femoral head articular cartilage of 3-month-old *Nfat1-/-* mice compared to age-matched WT mice. *Nfat1-/-* Ctl represents a negative control using both IL-1β antibody and IL-1β blocking peptide (Santa Cruz) to validate the specificity of the immune reaction. Scale bar = 200 μm. Modified from the published figure (Wang, et al. J Pathol 2009; 219:163-72) with permission from the publisher.

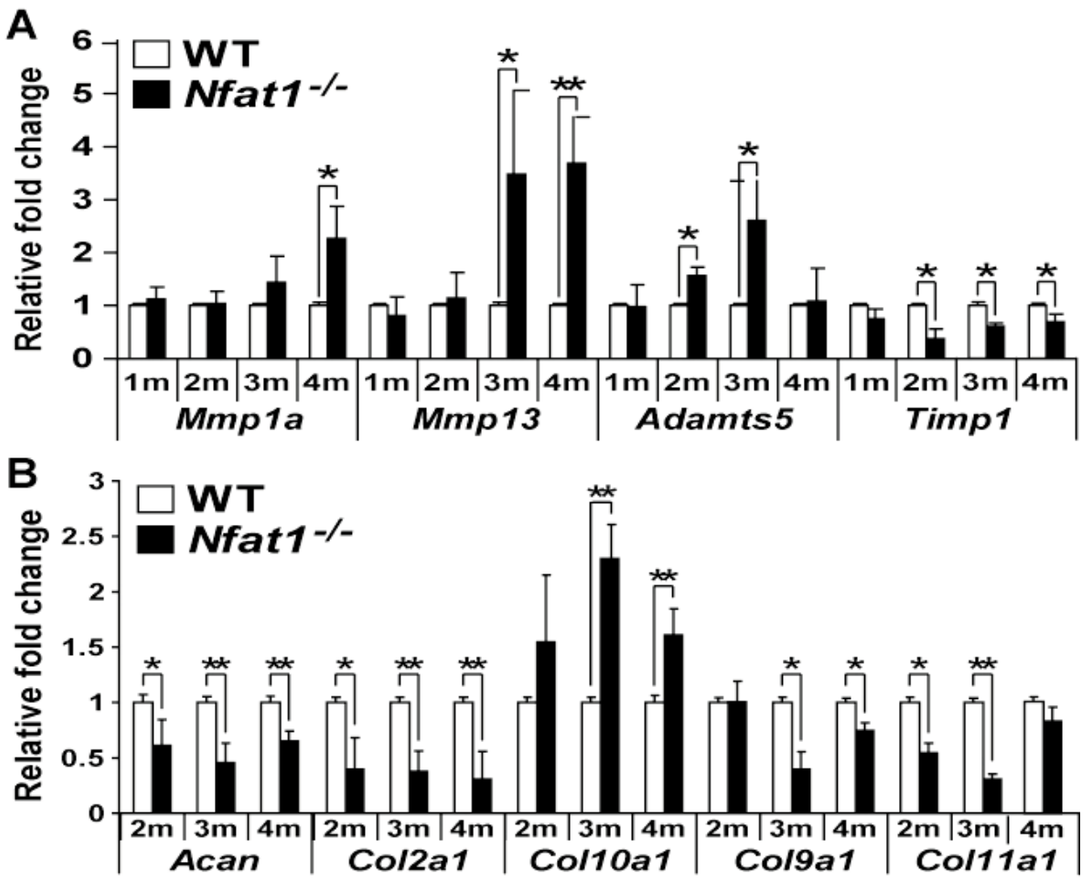

Fig. 3. Loss of Nfat1 leads to abnormal catabolic and anabolic activities of articular chondrocytes. (**A**) qPCR analyses demonstrate up-regulated expression of *Mmp1a, Mmp13, and Adamts5* and reduced expression of *Timp1* (tissue inhibitor of metalloproteinase-1) in femoral head articular cartilage of *Nfat1*$^{-/-}$ mice compared to age-matched WT mice at 1-4 months (1-4m) of age. (**B**) qPCR analyses indicate temporal changes in expression levels of various chondrocyte marker genes in *Nfat1*$^{-/-}$ articular cartilage at 2-4 months (2-4m) of age. The expression level of each WT group has been normalized to "one". n = 3 pooled RNA samples; * $P < 0.05$; ** $P < 0.01$. Modified from the published figure (Wang, et al. J Pathol 2009; 219:163-72) with permission from the publisher.

Thickening of subchondral bone is one of the characteristics of human OA. However, the precise biological mechanisms underlying the subchondral bone changes remain unclear. *Nfat1*$^{-/-}$ mouse joints display 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 differentiate into chondrocytes which subsequently underwent hypertrophy and endochondral ossification, leading to thickening of both subchondral plate and subchondral trabecular bone (Wang et al., 2009). These findings suggest that Nfat1 may prevent chondrocyte hypertrophy in adult articular cartilage and endochondral ossification in subchondral bone, thereby maintaining the integrity of cartilage-bone structure of synovial joints.

3.2.4 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).

A recent study demonstrated transcriptional activation of human MMP13 gene expression by c-Maf in osteoarthritic chondrocytes. C-Maf enhances MMP13 promoter activity and RNAi-mediated knockdown of c-Maf leads to a reduced expression of MMP13. Chromatin immunoprecipitation assays reveal that c-Maf binds to the MMP13 gene promoter, suggesting that MMP13 is a potential target of c-Maf in human articular chondrocytes (Li et al., 2010).

3.2.5 β-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-3β (GSK-3β). This interaction prevents GSK-3β from phosphorylating β-catenin to avoid degradation of β-catenin, thereby allowing β-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, β-catenin is a key mediator of the canonical Wnt signaling. Non-canonical Wnt signaling pathway, which does not involve β-catenin (Logan & Nusse, 2004), is probably best studied in *Drosophila* and its function is not covered in this chapter.

The canonical Wnt signaling pathway is known to induce chondrocyte maturation and endochondral ossification during skeletal development. This signaling pathway stimulates the commitment of mesenchymal stem cells to the preosteoblast and mature osteoblast phenotype, and blocks chondrogenic 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 tissue 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 β-catenin. Conditional ablation of β-catenin in chondrocytes leads to the absence of joints. Ectopic expression of activated β-catenin or Wnt-14 in chondrocytes leads to ectopic expression of joint markers (Tamamura et al., 2005; Hartmann & Tabin, 2001; Guo et al., 2004).

The canonical Wnt signaling is also important in susceptibility to OA. Recent studies revealed that Wnt/β-catenin is 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 β-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 expression (Zhu et al., 2009). Interestingly, the same group also reported that selective suppression of β-catenin signaling in articular chondrocytes also caused OA-like cartilage degradation in Col2a1-ICAT (inhibitor of β-catenin and T-cell factor) transgenic mice, and this was mediated by enhancement of apoptosis of the chondrocytes (Zhu et al., 2008). These results suggest that both excessive and insufficient β-catenin levels may impair the homeostasis of articular chondrocytes.

Transcription factors and transcriptional co-activators that may be responsible for the maintenance of the physiological function of adult articular chondrocytes and development of OA are presented in **Figure 4**.

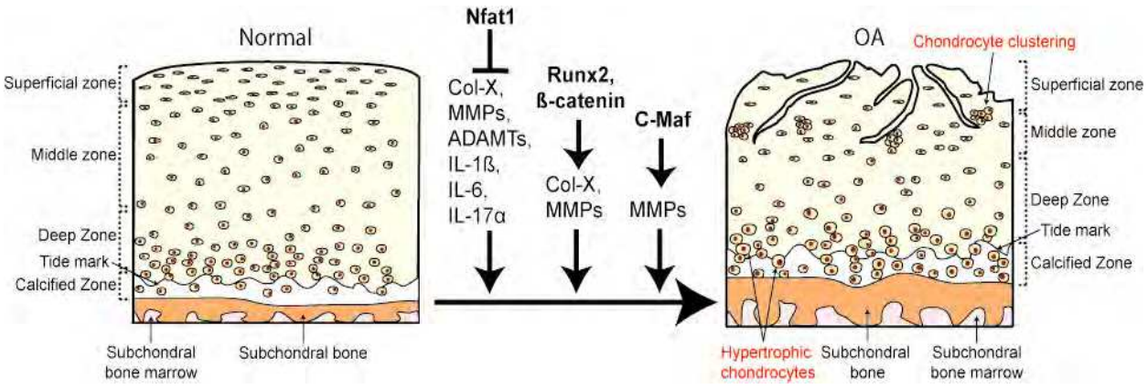

Fig. 4. A diagram demonstrates specific factors that may be responsible for the expression of catabolic molecules and collagen-X (Col-X, a marker of hypertrophic chondrocytes) during the development of OA.

3.3 Other factors that may regulate chondrocyte differentiation and function

3.3.1 BMPs

A variety of BMP members are present in articular cartilage. Among these BMPs, BMP-2 and BMP-7 (osteogenic protein-1, OP-1) are the two best studied BMPs with regard to cartilage homeostasis and OA. Both BMP-2 and BMP-7 have been shown to be capable of maintaining the chondrocyte phenotype. Inhibition of BMP-7 causes a reduction of aggrecan gene expression in chondrocytes. It has been reported that BMP-7 expression is decreased up to 9-fold in degenerated cartilage. In contrast to BMP-7, BMP-2 expression appears to increase with cartilage damage. In a study of BMPs in OA, BMP-2 is the only BMP member that demonstrates an increase in OA cartilage compared to cartilage from normal joints (Chubinskaya et al., 2007; Chubinskaya et al., 2000; Fukui et al., 2003; Sailor et al., 1996; Soder et al., 2005).

Multiple polymorphisms of the GDF-5 (BMP-14) 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).

3.3.2 Insulin-like growth factor-1 (IGF-1)

IGF-1 is expressed in normal articular cartilage and is generally thought to be an important growth factor for maintenance of articular chondrocyte phenotype and articular cartilage repair (Fortier et al., 2002; Fortier et al., 2011). Chronic IGF-1 deficiency causes an increased severity of OA-like articular cartilage lesions in rat knee joints (Ekenstedt et al., 2006). Human OA cartilage responds to IGF-1 treatment by increasing proteoglycan synthesis; however, catabolism in OA cartilage is insensitive to IGF-1 treatment (Morales, 2008).

3.3.3 Indian hedgehog (Ihh)

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 prehypertrophic chondrocytes and by early hypertrophic chondrocytes during endochondral ossification. Ihh knockout ($Ihh^{-/-}$) mice demonstrate abnormalities of chondrocyte differentiation and bone growth. Cartilage elements are small in $Ihh^{-/-}$ mice because of a marked decrease in chondrocyte proliferation. $Ihh^{-/-}$ 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. PTHrP, which is expressed by perichondral cells and early proliferative chondrocytes, down-regulates the expression of Ihh. This negative feedback loop mainly regulates the rate of chondrocyte differentiation and endochondral ossification. A third striking abnormality of $Ihh^{-/-}$ mice is the absence of osteoblasts in the primary spongiosa, suggesting that Ihh is required for osteoblast differentiation in endochondral bone formation (Kronenberg, 2003; Karp et al., 2000). Ihh is a critical and possibly direct regulator of joint development. In *Ihh*$^{-/-}$ mouse embryos, cartilaginous digit anlagen remained fused without interzones or mature joints (Koyama et al., 2007).
A recent study revealed that PTHrP may regulate articular chondrocyte maintenance in mice (Macica et al., 2011). In addition, parathyroid hormone (PTH) 1-34, a parathyroid hormone analog sharing the PTH receptor 1 with PTHrP, may inhibit the terminal differentiation of human articular chondrocytes *in vitro* and suppresses the progression of OA in rats (Chang et al., 2009).

3.3.4 Fibroblast growth factors (FGFs)

Recent evidence suggests that the 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 & 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).
The regulatory effects of FGF on adult articular chondrocytes are controversial in literature. A recent study showed that FGF-2 is an intrinsic chondroprotective agent that suppresses ADMTS5 and delays cartilage degradation in murine OA (Chia et al., 2009). However, other studies suggested that FGF-2 and FGF-23 may be involved in the progression of OA by stimulating MMP-13 expression through Runx2 (Orfanidou et al., 2009; Wang et al., 2004).

4. Future perspectives on pharmacological therapy

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 (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, c-Maf, β-catenin) may regulate 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. These findings have opened new avenues toward the development of DMOADs, using a more upstream factor as a molecular target than has been studied heretofore. In addition, 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. Therefore, other joint tissues should not be ignored when designing pharmacological therapies. Furthermore, insufficient recognition of pathological changes in 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 and treatment of OA may lead to the development of novel and effective therapeutic strategies using more up-stream pharmacological targets such as transcriptional signaling molecules, combined with biomechanical correction of abnormal joint loading if necessary, for the prevention and treatment of human OA.

5. Conclusion

OA is the most common form of joint disease and the major cause of chronic disability in middle-aged and older populations. All current pharmacological therapies are aimed at symptomatic control and have limited impacts on impeding or reversing the progression of OA, largely because the biological mechanisms of OA pathogenesis remain unclear. Previous studies have shown that overexpression of matrix-degrading proteinases and proinflammatory cytokines in articular cartilage is associated with osteoarthritic cartilage degradation. However, clinical trials applying an inhibitor of a proteinase or proinflammatory cytokine have been unsuccessful. 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 adult articular cartilage. This chapter summarizes the recent advances in the molecular regulation, with a main focus on transcriptional regulation, of the function of adult articular chondrocytes and its implication in the pathogenesis and treatment of OA. Recent studies have revealed that biological and mechanical abnormalities may affect transcriptional activity of specific transcription factors in articular chondrocytes. Transcription factor Sox9 is critical for the formation of cartilage, including articular cartilage, but its role in maintenance of adult articular chondrocyte function remains to be elucidated. Transcription factor Nfat1 plays 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 in their adult stage, including chondrocyte hypertrophy with overexpression of specific matrix-degrading proteinases and proinflammatory cytokines in articular cartilage. Transcription factor Runx2 and β-catenin transcriptional signaling may also be involved in the pathogenesis of OA via regulating the expression of anabolic and catabolic molecules in articular chondrocytes. These novel findings may provide new insights into the pathogenesis and treatment of OA.

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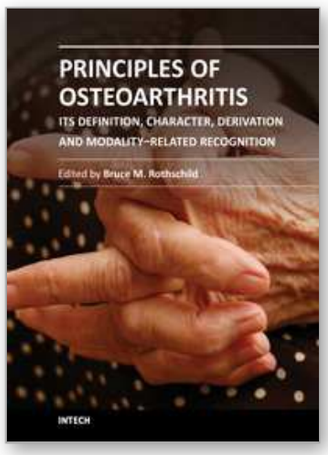

Principles of Osteoarthritis- Its Definition, Character, Derivation and Modality-Related Recognition

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This volume addresses the nature of the most common form of arthritis in humans. If osteoarthritis is inevitable (only premature death prevents all of us from being afflicted), it seems essential to facilitate its recognition, prevention, options, and indications for treatment. Progress in understanding this disease has occurred with recognition that it is not simply a degenerative joint disease. Causative factors, such as joint malalignment, ligamentous abnormalities, overuse, and biomechanical and metabolic factors have been recognized as amenable to intervention; genetic factors, less so; with metabolic diseases, intermediate. Its diagnosis is based on recognition of overgrowth of bone at joint margins. This contrasts with overgrowth of bone at vertebral margins, which is not a symptomatic phenomenon and has been renamed spondylosis deformans. Osteoarthritis describes an abnormality of joints, but the severity does not necessarily produce pain. The patient and his/her symptoms need to be treated, not the x-ray.

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