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REVIEW

Hypertrophic differentiation of chondrocytes in osteoarthritis: the developmental aspect of degenerative joint disorders

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Abstract

Osteoarthritis is characterized by a progressive degradation of articular cartilage leading to loss of joint function. The molecular mechanisms regulating pathogenesis and progression of osteoarthritis are poorly understood. Remarkably, some characteristics of this joint disease resemble chondrocyte differentiation processes during skeletal development by endochondral ossification. In healthy articular cartilage, chondrocytes resist proliferation and terminal differentiation. By contrast, chondrocytes in diseased cartilage progressively proliferate and develop hypertrophy. Moreover, vascularization and focal calcification of joint cartilage are initiated. Signaling molecules that regulate chondrocyte activities in both growth cartilage and permanent articular cartilage during osteoarthritis are thus interesting targets for disease-modifying osteoarthritis therapies.

Introduction

Osteoarthritis (OA) is the most common joint disorder in western populations. Its incidence increases with age, and thus this degenerative disease is a major problem in ageing populations. The disease is characterized by a progressive degradation of articular cartilage leading to loss of joint mobility and function accompanied by chronic pain. On the biochemical level, OA is characterized by uncontrolled production of matrix-degrading enzymes, including aggrecanases (a disintegrin and metalloprotease with trombospondine motifs (ADAMTSs)) and matrix metalloproteinases (MMPs), which result in the destruction of cartilage matrix [1]. Other hallmarks of the disease are new bone formation at the joint margins (osteophytes), limited inflammation (synovitis), and changes in subchondral bone structure (sclerosis). The molecular mechanisms regulating pathogenesis and progression of OA, however, are only poorly understood, and no proven disease-modifying therapy is currently available. Remarkably, some characteristics of OA – that is, articular chondrocyte proliferation, the expression of hypertrophy markers (for example, MMP-13 and collagen X), remodeling of the cartilage matrix by proteases, vascularization and focal calcification of joint cartilage with calcium hydroxyapatite crystals – resemble chondrocyte differentiation processes during skeletal development by endochondral ossification (EO). Signaling molecules regulating chondrocyte activities in growth cartilage may thus also be involved in OA pathogenesis.

In the present review, current concepts for the control of late chondrocyte differentiation in EO will be discussed in the light of analogous events observed during the development of OA. This knowledge is essential for the successful development of future therapeutic strategies.

Endochondral ossification

EO is important for development, growth, and repair of long bones. EO is initiated by the formation of cartilage templates of future bones, built by mesenchymal progenitor cells, which condensate and differentiate into chondrocytes. Within these bone anlagen, the differentiated cartilage cells then transit through a temporospatial cascade of late differentiation events that sequentially include proliferation and several steps of maturation, culminating in chondrocyte hypertrophy. After invasion of blood vessels from the subchondral bone, the majority of hypertrophic cells undergo apoptosis and the cartilage template is remodeled into trabecular bone [2]. Proliferation of chondrocytes, hypertrophic differentiation of chondrocytes, remodeling and mineralization of the extracellular matrix (ECM), invasion of blood vessels and apoptotic death of chondrocytes correspondingly occur during OA.

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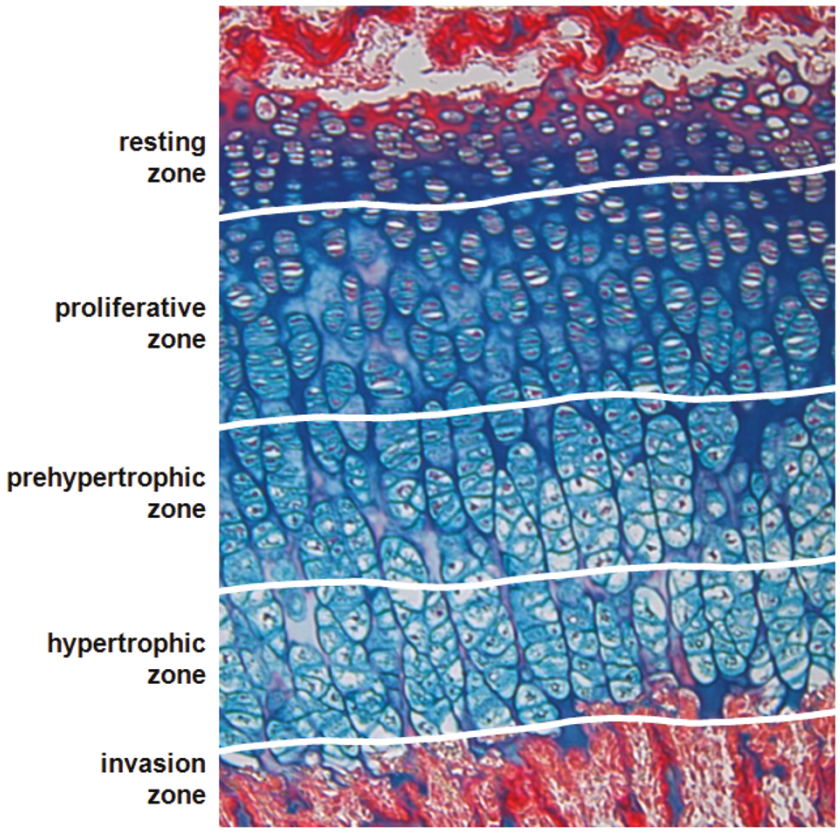

Figure 1. Organization of a 15-day-old murine tibia growth plate. Microphotograph of a Weigert's hematoxylin/alcian blue/sirius red stained section. Different growth plate zones can be distinguished according to changes in morphology and arrangement of the cells.

Each phase of EO is accompanied by a change in cell shape or cell arrangement [3,4] (Figure 1) and the expression of a specific protein repertoire. Collagen I, besides collagens III and V, is the major fibrillar component of undifferentiated mesenchymal progenitor cells [5]. After differentiation into chondrocytes, the cells cease to produce collagens I, III, and V but start to express typical cartilage components, including collagens II, IX, and XI and the proteoglycan aggrecan [6]. During this differentiation stage these so-called resting chondrocytes are small, uniform and characterized by low proliferation rates. These cells occur singly or in pairs, and in the resting zone the ECM takes more space than the cells. In the adjacent proliferative stage, the chondrocytes divide several times and the flat cells arrange into longitudinal columns. The expression repertoire now includes collagen VI [7] and matrilin 1 [8] in addition to the collagens II, IX and XI and aggrecan. During prehypertrophy, Indian hedgehog (Ihh) is expressed [9]. Further differentiation into hypertrophic chondrocytes induces the production of collagen X. Hypertrophic chondrocytes also reduce, or even terminate, their production of collagens II, IX, and XI, and express MMP-13, alkaline phosphatase, vascular endothelial growth factor (VEGF), osteopontin, and the transcription factor Runx2 [10]. Collagen X, MMP-13, and alkaline phosphatase are well-established markers for the overt hypertrophic stage of late chondrocyte differentiation.

Regulation of chondrocyte differentiation in growth cartilage

Chondrocyte differentiation in growth cartilage is subject to positive and negative control elements that interact within a signaling network to regulate the rate and progression of the process. EO is controlled by locally acting autocrine signals derived from chondrocytes themselves or by paracrine signals derived from cells of surrounding tissues (for example, the perichondrium or subchondral blood vessels). The interaction of chondrocytes with their surrounding matrix via cell surface receptors is also thought to play a key role in the regulation of survival, proliferation and maturation of

cartilage cells. Many stationary and diffusible regulators of chondrocyte differentiation as well as their cell surface receptors are proteins. Proteinases are thus not merely destructive effectors of ECM degradation but also intervene in regulatory networks, both by eliminating control elements (for example, endoplasmic reticulum protein 57 (ERp57) [11]) and by converting precursors into active agents (for example, transforming growth factor beta (TGFβ) [12]). In addition, proteinases modulate mediator activities by direct cleavage or by release from ECM stores (for example, VEGF [13]). Most signaling events culminate at the level of gene expression; thus transcription factors are also essential regulatory elements [10]. Several positive and negative feedback mechanisms exist, however, which complicate the signaling network.

Locally produced, secreted growth factors

Several locally produced factors – such as bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), TGFβ, Wnts, Ihh, parathyroid hormone-related peptide and retinoids – are so far known to influence EO. The actions of each of these growth factors during EO are briefly summarized below.

BMP signaling initiates chondroprogenitor cell differentiation, but in later EO stages induces chondrocyte proliferation and inhibits hypertrophic differentiation via Smad transcription factors [14]. Proteins of the FGF family, however, antagonize the BMPs. FGF-2 inhibits longitudinal bone growth by three mechanisms: decreased proliferation of growth plate chondrocytes, decreased cellular hypertrophy, and, at high concentrations, decreased cartilage matrix production. These effects may explain the impaired growth seen in patients with achondroplasia and related skeletal dysplasias [15]. FGF-2 inhibits chondrocyte hypertrophy in synergy with $TGFβ_2$ [16], while TGFβ alone inhibits chondrocyte proliferation, hypertrophy and mineralization [17]. In suspension cultures of chick sternum chondrocytes, TGFβ even initiates phenotypic changes of dedifferentiation [18]. On the other hand, $TGFβ_1$ has also been shown to increase alkaline phosphatase activity and to stimulate proliferation in rat costochondral cartilage cells through protein kinase C and protein kinase A signaling [19], suggesting a variability of TGFβ effects depending on the species, the differentiation status of the receiving cells and the TGFβ concentration.

Members of the Wnt family are involved in different stages of EO. During mesenchymal condensation, Wnt signaling favors osteoblastic differentiation but prevents chondrogenic differentiation; whereas at later stages, canonical Wnt/β-catenin signaling is indispensable for chondrocyte maturation. Wnt/β-catenin signaling acts as a positive regulator of chondrocyte hypertrophy and subsequent ossification [20]. Retroviral overexpression of Wnt9a (formerly known as Wnt14), one of the 19 ligands of the Wnt-signaling pathway, in chicken embryo limb buds results in a blockage of chondrogenic differentiation of the infected prechondrogenic region [21,22].

Ihh is expressed in the prehypertrophic chondrocytes of cartilage elements, where it regulates the rate of hypertrophic differentiation. In a feedback loop of paracrine control, perichondrial cells induced by the chondrocyte-derived Ihh produce parathyroid hormone-related peptide, which delays progression of late differentiation at late proliferative stages [9]. Additionally, proteins of the hedgehog family can also accelerate hypertrophic chondrocyte differentiation without involvement of parathyroid hormone-related peptide *in vitro* and *in vivo* [23], suggesting a direct effect of hedgehog proteins, possibly depending on the maturation stage of the receiving cell.

The vitamin A derivative retinoic acid positively regulates hypertrophic chondrocyte differentiation and matrix mineralization [24].

Hormones

In addition to locally produced growth factors, systemic hormones – such as growth hormone (GH), insulin-like growth factors (IGFs), thyroid hormone, androgen, estrogen and glucocorticoids – tightly regulate longitudinal bone growth. Local and systemic agents control the rate and extent of chondrocyte proliferation and differentiation at several checkpoints. This endocrine control enables longitudinal bone growth in healthy individuals and leads to increased growth rates and subsequent growth plate closure around puberty. GH and IGFs are potent stimulators of longitudinal bone growth. Both factors stimulate proliferation of resting zone chondrocytes and initiate chondrocyte hypertrophy [25]. Some effects of GH are likely to be mediated by IGF-I, with locally produced IGF-I seeming more important than systemic IGF-I [26].

Thyroid hormone is also indispensable in EO. Hypothyroidism slows down longitudinal bone growth, whereas hyperthyroidism accelerates the process. *In vitro* and *in vivo* studies have confirmed that thyroid hormone regulates the transition between cell proliferation and terminal differentiation in the growth plate; specifically, the maturation of chondrocytes into hypertrophic cells. Administration of thyroid hormone dose-responsively increases synthesis of type X collagen mRNA and protein, alkaline phosphatase activity, and cellular hypertrophy, all markers of the terminally differentiated phenotype of the growth plate chondrocyte [27].

Sex steroids are essential during the pubertal growth spurt and epiphysial fusion. Androgen stimulates chondrocyte proliferation and matrix production, and

thereby contributes to the increased long bone growth during the pubertal growth spurt. Estrogen affects growth plate cartilage through systemic as well as direct effects. On the one hand, estrogen regulates the GH/IGF-I axis, leading to decreased longitudinal growth and closure of the growth plate [28]; but estrogen also interacts with its receptors α and β within the growth plate, mediating direct effects [29].

The role of vitamin D signaling during bone development is well known because vitamin D deficiency leads to bone-softening diseases, such as rickets in children and osteomalacia in adults. These abnormalities result from decreased apoptosis of hypertrophic chondrocytes, widening of hypertrophic zones, and impaired bone mineralization. Some of the vitamin D effects are indirect through vitamin D actions on intestinal calcium and phosphate uptake. $24,25(OH)_2$ vitamin D_3, however, directly reduces chondrocyte differentiation in resting zone chondrocytes and stimulates late differentiation, while $1,25(OH)_2$ vitamin D_3 directly decreases proliferation and inhibits hypertrophic differentiation of proliferative cells through binding to a membrane-associated rapid-response steroid receptor (ERp57) on growth plate chondrocytes [11,30].

Extracellular matrix molecules

An intact fibrillar periphery is a prerequisite for normal cellular architecture of the growth plate, with collagen IX being particularly important for proliferation and maturation of chondrocytes. Newborn mice lacking collagen IX develop abnormal areas with strongly reduced cell numbers within the epiphysis of long bones. In addition, a disturbed columnar arrangement of chondrocytes was detectable, resulting in shorter and broader long bones especially in newborn collagen IX-deficient mice [31]. The importance of cell-matrix interactions also was demonstrated in mice deficient in receptor proteins on chondrocytes that interact with ECM molecules. Integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_{10}\beta_1$ are the major collagen receptors in cartilage. Their role in the spatial arrangement of growth plate chondrocytes and in outside-in-signaling has been intensively studied. Integrins, and their downstream signals integrin-linked kinase and the Rho GTPase Rac1, seem to act in a common pathway regulating cartilage development and disturbances in outside-in-signaling from the ECM to the cytoskeleton contribute to severe skeletal phenotypes [32-34].

Integrins are not the only ECM receptors in cartilage. The discoidin domain receptors (DDRs) are members of a subfamily of tyrosine kinase receptors that are activated by a number of different collagens, amongst others by collagens II and X. DDRs regulate cell proliferation, adhesion and motility, and control remodeling of the ECM by influencing the expression and activity of MMPs; for example, MMP-13 [35]. Mice lacking DDR2 exhibit dwarfism due to decreased proliferation of growth plate chondrocytes [36].

Proteases

MMPs are members of a family of zinc-dependent proteolytic enzymes. Several MMPs are expressed in bone and cartilage at high levels and are essential for normal EO. MMPs are directly involved in the degradation of proteins such as collagens and proteoglycans necessary for remodeling of the cartilaginous template during EO. Of further interest are the ECM degradation products, called matrikines, which are involved in the induction of higher concentrations or additional catabolic enzymes, amplifying the ECM remodeling. In addition to ECM degradation, however, several proteases are involved in the recruitment of cells into the growth plate through activation of recruitment factors (for example, VEGF) or influencing their bioavailability within the matrix [13].

Bone phenotypes are detectable in several mouse strains with MMP deficiencies. Although hypertrophic chondrocytes of MMP-9-deficient animals develop normally, apoptosis, vascularization, and ossification are delayed, resulting in progressive lengthening of the growth plate [37]. Wu and colleagues observed that MMP-13 activity is required for chondrocyte differentiation associated with matrix mineralization [38]. Studies on mice with compound inactivation of the MMP-9 and MMP-13 genes reveal that both proteases act in a synergistic manner. The double-null mice display severely impaired endochondral bone formation, characterized by diminished ECM remodeling, prolonged chondrocyte survival, delayed vascular recruitment and defective trabecular bone formation, resulting in drastically shortened bones [39]. MT1-MMP (MMP-14) deficiency causes a delay in the formation of the first and second ossification centers, a disorganized proliferation zone with reduced proliferation of chondrocytes, and an expanded zone of hypertrophic chondrocytes [40]. In addition the cysteine proteinases, especially the cathepsins, have also been implicated in several proteolytic scenarios during development, growth, remodeling, and aging. Throughout endochondral ossification, cathepsins B, H, K, L, and S were detected immunohistochemically in growth plates of rats and humans [41] and are thought to be involved in the proteolysis of several ECM components. We could show that cysteine proteinases mediate the shedding of $1,25(OH)_2$ vitamin D_3 membrane-associated rapid-response steroid receptor (endoplasmic reticulum protein 57), trigger chondrocyte size expansion and trigger expression of collagen X, alkaline phosphatase, and MMP-13 as markers for overt hypertrophy [11].

Transcription factors

Gene expression during distinct chondrocyte maturation phases within the epiphysial cartilage or growth plates is controlled by transcription factors translating the environmental signals into regulated gene expression. The two main transcriptional regulators of chondrogenesis and hypertrophic differentiation – Sox9 and Runx2 – should be addressed.

Sox9 was characterized as the master gene of chondrogenesis that regulates proliferation and differentiation of nonhypertrophic chondrocytes. Along with Sox5 and Sox6, Sox9 regulates the expression of aggrecan, and the α_1 chains of collagen II and collagen XI [42]. In addition, Sox9 acts as a negative regulator of chondrocyte hypertrophy, cartilage vascularization, and bone marrow formation [43].

Runx2 and its relative Runx3 are central positive regulators of the transition from proliferating to hypertrophic chondrocytes. Runx2/3 double-deficient mice were shown to lack hypertrophic chondrocytes anywhere in the skeleton [44]. In addition, Runx2-deficient mice lack upregulation of VEGF in hypertrophic chondrocytes and thus cartilage angiogenesis, suggesting that VEGF expression during bone development is controlled by the transcription factor Runx2 [45].

Another important transcription factor in bone development, detected recently, is CCAAT/enhancer-binding protein beta (C/EBPβ). C/EBPβ deficiency in mice was shown to cause dwarfism with elongated proliferative zones and delayed chondrocyte hypertrophy in growth cartilage [46]. Since growth arrest and DNA damage (GADD)45β$^{-/-}$ animals [47] display similar phenotypes to the C/EBPβ knockout mice [46], the molecular interplay of GADD45β and C/ERBβ was analyzed in further detail. Various experiments indicate that GADD45β enhances C/ERBβ transactivation of the collagen 10a1 promoter and therefore is an upstream modulator of C/EBPβ [48].

Chondrocyte differentiation processes in articular cartilage

Articular cartilage is formed for life. Articular chondrocytes therefore display only moderate metabolic activity under normal conditions, primarily to maintain their surrounding ECM comprising collagens (collagens II, VI, IX and XI), proteoglycans (aggrecan, decorin, biglycan and fibromodulin) and further noncollagenous matrix proteins. Under nondiseased conditions, the cells remain in a resting state and refrain from proliferation or terminal differentiation. In a diseased state, however, some articular chondrocytes lose their differentiated phenotype; they enter an EO-like cascade of proliferation [49] and hypertrophic differentiation, accompanied by marker expression for the overt hypertrophic differentiation stage, such as alkaline phosphatase [50], collagen X [51], and MMP-13 [52], with subsequent apoptotic death [53] and mineralization of the diseased cartilage [54] (Figure 2).

OA is considered a multifactorial disease; however, the scenario that OA is, at least in part, based on illegitimate hypertrophic differentiation should be taken into account. Differentiation of chondrocytes leads to an enhanced metabolic activity of articular chondrocytes, a change in the expression of ECM molecules, and an altered pattern of proteases. Altogether, this differentiation triggers a disturbed cartilage homeostasis favoring degenerative changes. Several signaling factors involved in chondrocyte proliferation and differentiation during endochondral ossification were also shown to play a regulative role in OA cartilage, but not under nondiseased conditions. In all cases in which this signaling initiates the modulation of ECM or the expression or activation of proteases (for example, MMP-13 or aggrecanases), differentiation changes should be considered a potential driver of OA. A number of examples illustrating the analogy of signaling events in bone development by EO and cartilage degeneration in OA are given below. The reader should, however, keep in mind that most of the factors reviewed here were analyzed in spontaneous, transgenic or surgically induced mouse models of OA but not in large animals, which occasionally better reflect human OA pathophysiology.

Growth factor signaling in osteoarthritis

Chondrocyte differentiation and matrix remodeling in osteoarthritic cartilage is regulated by BMPs, FGF-2, TGF-β, Wnts, hedgehogs and retinoids – all of which are also involved in the regulation of EO.

Although BMP-2 has potent anabolic actions, BMP activity in chondrosarcoma cells and in murine cartilage was shown to induce OA-like changes by stimulation of MMP-13 [55], directly favoring cartilage loss. FGF-2, however, displays beneficial effects on articular cartilage homeostasis. Chia and colleagues observed that FGF-2-deficient mice had increased OA development with age as compared with wild-type mice. This is due to an increased expression of ADAMTS-5, the key murine aggrecanase [56]. The contrary role of BMP and FGF signaling pathways in OA was also described recently in respect of extracellular heparan sulfatases Sulf-1 and Sulf-2, which are found overexpressed in OA cartilage. Sulfs simultaneously enhance BMP signaling via Smad1/5 phosphorylation but inhibit FGF signaling via ERK1/2 phosphorylation, and thereby maintain cartilage homeostasis and favor cartilage repair [57].

Lack of TGFβ signaling results in OA-like changes with terminal differentiation of chondrocytes. As shown in genetically modified mice, TGFβ mediates this effect by

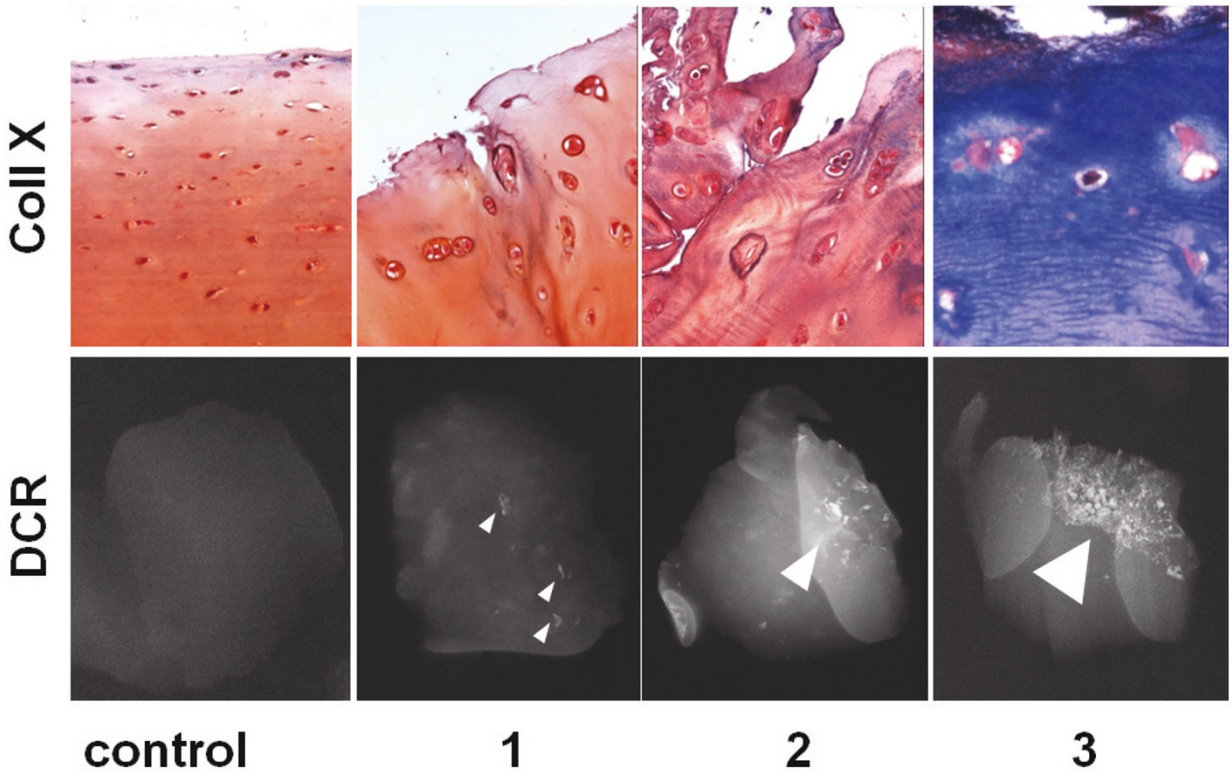

Figure 2. Knee cartilage showing increased hypertrophy and mineralization. Knee cartilage with higher Mankin scores shows increased hypertrophy (increase in collagen X staining; fast blue) and mineralization (arrowheads). Histologic assessment of the knee was performed on cartilage plugs from the medial femoral condyle, and a modified Mankin scoring system was used to assess the severity of changes in osteoarthritis (OA) articular cartilage. The Mankin grades (range 0 to 14 points) for mild (1), moderate (2), and severe (3) OA were 2 to 5, 6 to 9, and 10 to 14 points, respectively. To assess hypertrophic differentiation, collagen X staining was performed on paraffin sections after hyaluronidase (Sigma, Taufkirchen, Germany) digestion with collagen X antibody (ab58632; Abcam, Cambridge, UK) using the Vectastain ABC Elite Kit (Vector Laboratories, Peterborough, UK) and fast blue as a chromogen. To assess mineralization, the articular cartilage was analyzed using digital contact radiography (DCR), performed using a digital mammography imaging technique (Hologic, Waltham, MA, USA) operating at 25 kV in manual mode, usually at 3.8 mA, and with a film focus distance of 8 cm. See [54]. Photographs kindly provided by Martin Fuerst, University Medical Center Hamburg-Eppendorf.

binding to the ALK5 type-I TGFβ receptor and subsequent activation of the Smad2/3 intracellular signaling route [17,58]. Notably, TGFβ supplementation can enhance cartilage repair and therefore was thought to serve as a potential therapeutic tool. Conversely, supplementation with TGFβ provides problems in non-cartilaginous tissues of the joint and results in fibrosis and osteophyte formation in a murine model of OA [59]. Moreover, Van der Kraan and colleagues recently suggested a dual role for TGFβ in articular mouse cartilage because not only signaling via ALK5 (Smad2/3) but also via ALK1 (Smad1/5/8) can be initiated by TGFβ. Importantly, only signaling via ALK1, but not via ALK5, stimulates MMP-13 expression and thereby collagen degradation [60].

Recent genetic data of Caucasian test persons linked a polymorphism in the FrzB gene, encoding for a Wnt binding protein, to the development of OA, suggesting that abnormal Wnt signaling also contributes to OA [61]. Blom and colleagues found that β-catenin, along with a panel of other Wnt/Fz-related genes, was upregulated in cartilage and synovium during experimental OA in mice. The authors identified WISP-1 (capable of inducing cartilage-degrading enzymes such as MMPs, ADAMTS-4 and ADAMTS-5), independently of the catabolic cytokine IL-1, as a crucial Wnt signaling mediator [62].

Moreover recent studies using a transgenic OA mouse model with conditional activation of the β-catenin gene in articular chondrocytes showed that upregulation of β-catenin signaling is most probably responsible for the conversion of normal articular chondrocytes into arthritic, OA-like cells. Chondrocyte maturational genes were activated along with the induction of matrix degradation [63,64]. Hedgehog signaling was also described to play a role in OA. Lin and colleagues demonstrated an increased expression of hedgehog targets in human OA samples and mouse articular cartilage after surgical OA induction. Amplified hedgehog target gene

expression correlated with advanced disease stages, and hedgehogs were described to stimulate the expression of the aggrecanase ADAMTS-5 via the transcription factor Runx2 [65].

Last, but not least, the retinoids display multiple effects relevant to the OA disease process. Davies and colleagues showed that components of the retinoid signaling pathways are upregulated during OA in humans and that all-*trans*-retinoic acid treatment of human explant cartilage samples led to significant increase of MMP-13 and aggrecanases, enzymes involved in two of the key proteolytic processes implicated in OA [66]. Taken together, many locally acting growth factors playing a crucial role in the regulation of chondrocyte proliferation and differentiation during EO also are involved in OA pathogenesis and disease progression, mainly by stimulation or activation of degradative enzymes (for example, MMP-13 or aggrecanases).

Role of hormones in osteoarthritis

A number of different studies have attempted to link changes in hormonal status to the pathogenesis or progression of OA, but in the majority of cases inconsistent results were obtained. The prevalence of knee OA, for example, is increased among woman after the age of 50 years, and this phenomenon has been ascribed to estrogen insufficiency. No clear association has yet been found, however, between hormone deficiency and OA of the hand, hip and knee [67]. Reports about estrogen replacement therapies and their outcome on OA incidence also show inconsistent results [68]. In addition to estrogen, the vitamin D status is unrelated to the risk of joint space or cartilage loss in knee OA [69]. Moreover, no association was found between vitamin D receptor polymorphisms and OA susceptibility in a large meta-analysis [70].

With respect to the GH/IGF-I axis, however, it was shown in a rat model of OA that chronic GH deficiency causes an increased severity of articular cartilage lesions of OA, although the IGF-I expression is increased [71]. Anabolic IGF-I signaling is antagonized by increased occurrence of IGF-binding proteins, which then negatively regulate IGF-I signaling in chondrocytes during OA [72]. In contrast, patients with GH deficiency had significantly less OA than the normal patients of a control population, suggesting GH to be a beneficial factor in the development of OA [73].

Role of extracellular matrix molecules in osteoarthritis

Cell-matrix interactions are essential regulators, both in EO and OA. One such example is deficiency in the collagen IX α_1 chain, a perifibrillar component of cartilage fibrils, containing collagens II and XI. Knockout animals were not only shown to have a growth plate phenotype but also to develop a severe degenerative joint disease resembling human OA [74,75]. Histological analysis reveals OA-like changes in an age-dependent manner in the knee and temporomandibular joints starting at the age of 3 months. Later, at the age of 6 months, enhanced proteoglycan and collagen degradation due to higher expression of MMP-13 was observed. The FACIT collagen IX is directly and indirectly involved in the mutual interaction of the extrafibrillar matrix components with cartilage fibrils [76]. The disturbed tissue integrity possibly triggers a higher susceptibility of collagen IX α_1 knockout animals to cartilage degradation.

Furthermore, as in EO, matrix receptors binding to the structural components of the ECM have an impact on chondrocyte behavior in articular cartilage. One such example is the deficiency of the α_1 subunit of integrins, which in mice was detected to be associated with an accelerated, aging-dependent development of OA [77]. Mice with a conditional deletion of the β_1-integrin gene in early limb development using a mitochondrial peroxiredoxin Prx1-cre transgene showed multiple abnormalities of knee-joint articular cartilage, accompanied by accelerated terminal differentiation. The cartilage homeostasis in these mice, however, was comparable with wild-type animals, suggesting a minor importance of signaling events mediated through integrins in cartilage destruction [78].

Other examples affecting chondrocyte behavior, however, are the DDR receptors. Xu and colleagues detected that increased expression of the collagen receptor DDR-2 in articular cartilage represents a key event in the pathogenesis of OA [79]. The authors not only describe increased immunostaining for DDR-2 but also for MMP-13 and MMP-derived type II collagen fragments in cartilage from patients with OA and from mice with surgically induced OA, and they linked the enhanced MMP-13 expression by mutation analysis directly to enhanced DDR-2 signaling. Based on these observations, they hypothesized that exposure of the type II collagen network to chondrocytes results in enhanced contact of the cells with type II collagen fibrils. DDR-2 is activated as a consequence of the interaction of type II collagen with chondrocytes, resulting in the increased expression of the receptor itself as well as MMP-13. Increased expression of DDR-2 may thus be a common event in the pathogenesis of OA in general [79].

Role of proteases in osteoarthritis

Proteolytic degradation of articular cartilage involving the key players of the MMP family, the a disintegrin and metalloprotease (ADAM) family and the ADAMTS protease family is a central event during OA. The structural integrity of the ECM is damaged by these enzyme activities, and cell-matrix interactions influencing chondrocyte activities are destroyed.

Table 1. Different signaling factors involved in both chondrocyte differentiation processes during endochondral ossification and in osteoarthritis

Signaling factor	Effects on growth plate chondrocytes	Role in OA
Bone morphogenic proteins	Induce proliferation	Stimulation of MMP-13
	Inhibit hypertrophy	
Fibroblast growth factors	Decrease proliferation	Stimulation of ADAMTS-5
	Decrease hypertrophy	
	Decrease matrix production	
TGFβ	Variable effects depending on species and concentration	Stimulation of MMP-13
Wnt/β-catenin	Positive regulator of hypertrophy and ossification	Activation of maturational genes
		Induction of matrix degradation
		Induction of MMPs and aggrecanases mediated by WISP-1
Indian hedgehog	Stimulates proliferation	Induction of ADAMTS-5 via Runx2
	Inhibits hypertrophy via parathyroid hormone-related peptide	
	Directly induces hypertrophy *in vitro*	
Retinoic acid	Positive regulator of hypertrophy and matrix mineralization	Induction of MMP-13 and aggrecanases
Growth hormone/IGF-I	Stimulate proliferation	Growth hormone is a beneficial factor in OA
	Initiate hypertrophy	IGF-I signaling is antagonized by IGF-binding proteins
Collagen IX	Stimulates chondrocyte proliferation	Essential for tissue integrity, loss of collagen IX induces OA
	Essential for columnar organization of growth plate chondrocytes	
β_1 integrins	Mediate adhesion to surrounding matrix and motility	Essential for normal knee joint development
	Essential for proliferation	Minor influence on cartilage homeostasis
Discoidin domain receptors	Regulate cell proliferation, adhesion and motility	Induction of MMP-13 and MMP-derived type II collagen fragments
	Control matrix remodeling	
MMPs/ADAMTSs	Essential for matrix remodeling	Key factors in matrix degradation during OA
	Influence bioavailability of VEGF	Matrix degradation is accompanied by terminal chondrocyte differentiation, positive feedback mechanism?
Sox 9	Regulate proliferation and hypertrophic differentiation	Involved in MMP-13 expression
Runx2/3	Positive regulation of chondrocyte hypertrophy	Induction of chondrocyte hypertrophy
	Influence on angiogenesis by upregulation of VEGF	Induction of MMP-13 expression
CCAAT/enhancer binding protein beta	Inhibition of proliferation	Mediates cartilage destruction
	Stimulation of hypertrophy	
	Activation of collagen X expression	

Note that cartilage degradation in osteoarthritis (OA) is mediated by matrix metalloproteinase (MMP)-13 and aggrecanases (a disintegrin and metalloprotease with trombospondine motifs (ADAMTS)-4 and ADAMTS-5) expressed by hypertrophic chondrocytes. See relevant references in the text. IGF, insulin-like growth factor; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor.

Although ADAMTS-4 expression is upregulated in human OA, only the lack of ADAMTS-5 prevented cartilage degradation in a mouse model of surgically induced OA [80]. Remarkably, the heparan sulfate proteoglycan syndecan-4 regulates ADAMTS-5 activation and cartilage breakdown in murine OA through direct interaction with the protease and through regulating mitogen-activated protein kinase-dependent synthesis of MMP-3 [81]. To investigate the role of MMP-13 in cartilage degradation and chondrocyte differentiation during OA, Little and colleagues surgically induced OA in the knees of MMP-13-deficient and wild-type mice. These authors observed that MMP-13 deficiency can inhibit cartilage erosion but not chondrocyte hypertrophy or osteophyte generation during OA, suggesting that chondrocyte hypertrophy is accompanied by, but not directly regulated by, MMP-13 [82]. Tchetina and colleagues investigated the interrelationship between the extent of collagen cleavage by collagenases and the expression of differentiation-related genes [83]. These

authors detected that early focal cartilage degradation by MMP-1, MMP-14 and ADAMTS-5 was accompanied by the expression of terminal differentiation-related genes COL10A1, MMP-13, MMP-9, Ihh and caspase 3, suggesting that chondrocyte differentiation may be closely related to the very early development of cartilage degeneration. Taken together these results indicate that matrix remodeling processes show similar characteristics in EO and OA.

Role of transcription factors in osteoarthritis

Orfanidou and colleagues recently investigated the involvement of the most fundamental transcription factors in EO – Runx2 and Sox9 – in the regulation of osteoarthritic chondrocytes. The authors demonstrated convincing associations among Runx2, Sox9 and FGF-23 in relation to MMP-13 expression in osteoarthritic chondrocytes, contributing to the cartilage degeneration process [84]. Kamekura and colleagues surgically induced OA in Runx2$^{+/-}$-deficient mice and wild-type mice. The heterozygous Runx2-deficient mice exhibited decreased cartilage destruction and osteophyte formation, along with reduced type X collagen and MMP-13 expression, as compared with wild-type mice – suggesting a contribution of the transcription factor Runx2 to the pathogenesis of OA through chondrocyte hypertrophy and matrix breakdown after the induction of joint instability [85]. Taken together, these two major transcriptional regulators of chondrogenesis and hypertrophic differentiation (Sox9 and Runx2) play a role not only in bone development by EO but also in cartilage degradation in OA.

The transcription factor C/EBPβ was shown to directly transactivate p57^{Kip2} to promote the transition from proliferation to hypertrophic differentiation of chondrocytes and to influence the collagen type X expression during bone development [46,48]. This transcription factor mediates cartilage destruction during OA progression, since C/EBPβ$^{+/-}$ mice were protected against cartilage degradation in knee joints in an OA model [46]. Whether GADD45β is an upstream modulator of C/EBPβ in this instability-induced OA in mice, as was shown in chondrocyte terminal differentiation, needs to be shown in future experiments.

Conclusions

A number of signaling factors involved in chondrocyte proliferation and differentiation during EO were also shown to play a regulative role in articular cartilage during OA, pointing towards analogous signaling events that are critical for both scenarios (Table 1). All events leading to a structurally altered ECM in articular cartilage – for example, reduction in cartilage collagen production or induction of degradative enzymes – have to be taken into account as the driving force in the pathogenesis or progression of OA. Future work is necessary to investigate both of these processes in further detail in order to take advantage of the understanding of developmental aspects for pathogenetic mechanisms of degenerative joint disorders, and hence the successful development of future therapeutic strategies.

Abbreviations

ADAMTS, a disintegrin and metalloprotease with trombospondine motifs; BMP, bone morphogenic protein; C/EBPβ, CCAAT/enhancer binding protein beta; DDR, discoidin domain receptor; ECM, extracellular matrix; EO, endochondral ossification; FGF, fibroblast growth factor; GADD, growth arrest and DNA damage; GH, growth hormone; IGF, insulin-like growth factor; Ihh, Indian hedgehog; IL, interleukin; MMP, matrix metalloprotease; OA, osteoarthritis; Smad, Sma and Mad related proteins; Sulf, heparan sulfate 6-***O***-endosulfatase; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor.

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Competing interests

The author declares that she has no competing interests.

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