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Review

Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration?

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SUMMARY

Objective: To review the literature on the role and regulation of chondrocyte terminal differentiation (hypertrophy-like changes) in osteoarthritis (OA) and to integrate this in a conceptual model of primary OA development.

Methods: Papers investigating chondrocyte terminal differentiation in human OA cartilage and experimental models of OA were recapitulated and discussed. Focus has been on the occurrence of hypertrophy-like changes in chondrocytes and the factors described to play a role in regulation of chondrocyte hypertrophy-like changes in OA.

Results: Chondrocyte hypertrophy-like changes are reported in both human OA and experimental OA models by most investigators. These changes play a crucial part in the OA disease process by protease-mediated cartilage degradation. We propose that altered chondrocyte behavior and concomitant cartilage degradation result in a disease-amplifying loop, leading to a mixture of disease stages and cellular responses within an OA joint.

Conclusion: Chondrocyte hypertrophy-like changes play a role in early and late stage OA. Since not all cells in an OA joint are synchronized, inhibition of hypertrophy-like changes might be a therapeutic target to slow down further OA progression.

Introduction

Osteoarthritis (OA) is the most common joint disease and highly associated with age. This disease is characterized by a change in chondrocyte behavior that leads to elevated production of proteolytic enzymes, such as matrix metalloproteinase13 (MMP13), and consequently to cartilage damage and loss of joint function. However, one has to keep in mind that alternative routes are proposed that can lead to OA, amongst others mechanical wear and tear, bone changes and changes in the cartilage extracellular matrix.

Changes in chondrocyte behavior, such as expression of hypertrophy markers and matrix calcification, frequently resemble the phenomena observed in the hypertrophic layer of the growth plate of long bones, but other phenotypic changes are also reported[1]. In this article, we will discuss the occurrence and role of chondrocyte hypertrophy-like changes in (experimental) OA. Discussed will be cartilage calcification, apoptosis, autophagy, transgenic and knock out mouse models, and factors involved in regulation of hypertrophy-like changes. Finally, we will put forward a theoretical model of OA development and the part that hypertrophy-like changes play in this disease.

Chondrocyte differentiation

Hyaline cartilage can be largely divided into temporary and permanent cartilage. Temporary hyaline cartilage is found in the embryonic stages of endochondral bones and the growth plate and is finally replaced by bone. Permanent hyaline cartilage, present amongst others in articulating joints, does not undergo terminal differentiation under normal conditions. The cellular origin of both types of temporary and permanent cartilage is similar. As concluded from both *in vivo* and *in vitro* studies, the default route of chondrocyte differentiation is terminal differentiation (hypertrophy and apoptosis)[2–7]. An enlightening *in vivo* model of adult chondrocyte differentiation is osteophyte formation[8]. Osteophytes are the result of chondrogenic differentiation of mesenchymal stem cells in the periosteum. Initially cartilage is formed but the final product is bone. Apparently, unrestricted differentiation of precursor cells into the chondrocyte lineage does not lead to permanent cartilage but to bone. In articular cartilage, this default route is somehow blocked to obtain permanent cartilage.

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Chondrocytes in OA cartilage show an aberrant phenotype and actively produce cartilage-degrading enzymes, such MMP13 and aggrecanases[9–13]. The exact activation mechanism of chondrocytes in OA cartilage is not understood. Age-related changes in the extracellular matrix molecules or activation by inflammatory cytokines, like IL-1, might play a role in direct activation of the chondrocytes as recently discussed[14–16]. Alternatively, the altered phenotype might be the result of articular chondrocytes taking a differentiation route resembling that of growth plate chondrocytes, expressing hypertrophy-like changes. This will be accompanied by acquisition of an autolytic phenotype by the chondrocytes resulting in destruction of the surrounding cartilage. The occurrence of chondrocyte hypertrophy-like changes in OA is discussed below.

Indications for chondrocyte hypertrophy-like changes in OA

The exact pathogenic mechanism of OA is still not elucidated, if there is at all a general, major mechanism. It can be anticipated that more than one road leads to cartilage destruction and loss of joint function. This can be different for different OA subgroups. A route that has been proposed is that articular chondrocyte loose their differentiated phenotype and obtain a behavior with similarities to terminal differentiating chondrocytes (hypertrophy-like), as can be found in the growth plate of growing individuals[17,18].

Markers of hypertrophic chondrocytes are several, the most widely used are type X collagen and MMP13[19–21]. However, osteopontin, osteocalcin, Indian Hedgehog, Runx2, VEGF, HtrA1 and transglutaminase-2 (TG-2) are all shown to be associated with chondrocyte hypertrophy[22–28]. However, one has to keep in mind that, although accepted as a hypertrophy marker, MMP13 synthesis is induced in chondrocytes by alternative routes, such as inflammation and mechanical stress[16,29,30].

The ultimate end stage of chondrocyte hypertrophy is calcium deposition. This state has not necessarily to be reached in OA cartilage. More important for the disease initiation and progression is the fact that hypertrophic chondrocytes degrade their surroundings, as can be seen in OA. What is more, cartilage calcification does not have to be unavoidably related to chondrocyte hypertrophy. However, calcification of articular cartilage appears to be associated with elevated expression of hypertrophy markers[31].

Calcification of articular cartilage

Calcification of articular cartilage and ossification as a result of terminal differentiation of chondrocytes might be different processes, but a number of studies have indicated overlapping phenomena. Studies in the sixties and seventies of the last century have shown that in human cartilage the number of tidemarks, the line bordering the calcified cartilage and the non-calcified cartilage, is increased above the age of 60[32,33]. This movement of the tidemark to the cartilage surface should result in an increase in the thickness of the calcified cartilage, but it is shown that instead the calcified zone becomes thinner during aging. This indicates replacement of the lower calcified cartilage by bone because of endochondral ossification.

Not only aging, but also OA is related to ossification processes in articular cartilage. Next to increased alkaline phosphates and pyrophosphate levels, calcification is a common observation in OA[34–38]. Not only in large joints, but also degenerative changes in intervertebral discs have been associated with tissue calcification[39,40]. Articular cartilage calcification is not always easily identified by X-ray, but Fuerst *et al.* showed the presence of calcifications in all examined OA cartilage samples by digital contact radiography[31]. In these studies, severity of OA correlated positively with the amount of matrix calcification and basic calcium phosphate was identified as the principal mineral. In cell culture experiments, OA chondrocytes showed a strong correlation between their ability to produce calcium phosphate *in vitro* and the extent of mineralization observed in the donors. Furthermore, induction of hypertrophy-like changes in healthy human chondrocytes led to unambiguous calcification of the extracellular matrix[31,41,42].

However, others have concluded that calcification of OA articular cartilage is related to age instead of OA itself[43]. This is mainly based on the observation that no further increase in cartilage calcium levels was observed when OA structural changes increased from moderate to severe. From these data, it could also be concluded that calcification is a rather early phenomenon and does not further increase in end-stage OA. In addition, age is the main risk factor for OA development and age-related calcification points to an age-related underlying mechanism, coupling calcification and cartilage damage.

Markers for chondrocyte hypertrophy-like changes in human cartilage

Type X collagen is considered as the standard marker for chondrocyte hypertrophy. The function of type X collagen is still not clear but its expression at sites of chondrocyte hypertrophy and calcification suggest a role in the early stage of endochondral bone formation. Type X collagen is normally not expressed in human healthy articular cartilage but its expression is detected at protein and mRNA level in human OA cartilage[17,44–50]. Particularly in the vicinity of lesions, COL10A1 has been shown to be up regulated[51]. In a study of Fukui *et al.*, studying the regional and zonal differences in gene expression in OA cartilage, it was demonstrated that overall expression of COL10A1 was elevated but that expression showed significant local variation. Notably, the expression of type COL10A1 was higher in the less degenerated than in the more degenerated areas[52,53]. However, a number of expression array studies, both in human and experimental OA, have failed to detect elevated COL10A1 expression in OA cartilage[47,48,54]. Sampling differences, zonal differences and stage dependent alterations might explain these differences. An alternative explanation is that chondrocyte hypertrophy-like changes are not a general phenomenon in human OA but only observed in a subgroup of human OA patients.

Another marker of hypertrophy, collagenase-3 or MMP13, is also frequently shown to be elevated in OA cartilage[9,10,55,56]. Up regulation of MMP13 by more than 40-fold in OA cartilage compared to normal cartilage was shown by microarray and RT-PCR[52]. Moreover, even investigators that did not report an elevation of COL10A1 expression clearly demonstrated increased expression of MMP13 in OA cartilage[48,57], which appears to indicate that MMP13 and type X collagen expression are uncoupled and MMP13 expression can be elevated by alternative pathways than chondrocyte terminal differentiation.

Other genes or proteins, related to chondrocyte hypertrophy, that have been demonstrated to be more highly expressed in OA cartilage than normal cartilage are amongst others; osteocalcin[50], osteopontin[58], osteonectin[59], Indian Hedgehog[51], Runx2[60], VEGF[61], beta-catenin[62], alkaline phosphatase[59]. Expression of Sox9, a crucial transcription factor for chondrocytes, is down regulated in human OA cartilage as is observed in terminal differentiating growth plate chondrocytes[48,60,63,64].

Hypertrophic chondrocytes in the growth plate do not only express specific molecules such as type X collagen, but also undergo apoptosis to make bone deposition possible[65]. Apoptosis can be considered as a marker for chondrocyte hypertrophy in OA cartilage[66]. Chondrocytes in human OA cartilage have demonstrated elevated Tunel staining and express the apoptosis markers,

annexin II and V, caspase-3 and -9, BCL-2 and FAS[49,67–69]. Moreover, caspase inhibitors reduce severity of cartilage lesions in experimental models of OA[70]. However, the role of apoptosis in OA remains enigmatic. Some studies show such a high number of apoptotic cells that this is hard to merge with a slowly progressive disease. Nevertheless, the abundant presence of apoptosis markers in OA chondrocytes suggests a phenotype that resembles that of terminally differentiated hypertrophic chondrocytes.

A process related to apoptosis and that has attracted increasing attention in OA is autophagy. Hypertrophic chondrocytes in the growth plate have been shown to undergo autophagy[71]. Autophagy has been put forward as an alternative cause of chondrocyte cell death in OA[72]. In contrast, autophagy could be a cartilage protective mechanism which is lost upon aging and OA development[73,74]. The exact role of autophagy in chondrocyte hypertrophy and OA is not elucidated yet but will be an important subject of investigation in the coming years.

Markers for chondrocyte hypertrophy-like changes in OA models

Little is known about chondrocyte hypertrophy and spontaneous age-related OA in animal models. However, increased expression of chondrocyte hypertrophy markers has been observed in OA cartilage of aging OA-prone Hartley guinea pigs and horses with OA show chondrocyte apoptosis[25,75,76]. Somewhat more information is available about the occurrence of these markers in induced OA models. Hypertrophy markers have been observed in several models of OA in different species. In mice, in which OA was induced by provoking knee joint instability by transection of knee ligaments and removal of the medial meniscus, elevated expression of MMP13 and type X collagen was reported[77,78]. Rats that underwent anterior cruciate transection and forced mobilization expressed alkaline phosphatase, MMP13 and type X collagen in the affected knee joints[79]. Medial meniscectomy in larger animals such as sheep resulted in chondrocyte hypertrophy-like changes in the OA cartilage after 6 months[80]. These observations indicate that chondrocyte hypertrophic changes in OA cartilage are not limited to humans and can be observed in both small and large animals. In mice, regulation of chondrocyte hypertrophy and its relation with OA development have been studied in a large number of genetically-modified animals.

Regulators of chondrocyte hypertrophy-like changes and OA

A number of transgenic mouse strains, both knock outs and transgenics, have been developed that support a role for chondrocyte terminal differentiation in the OA process. Genetic modifications that stimulate chondrocyte hypertrophy-like changes are frequently associated with a higher incidence of OA or accelerated OA development. However, one has to keep in mind that many of these genetic alterations might also affect bone and joint formation and influence in this way OA development. Moreover, the observation that genetic modifications drive chondrocyte hypertrophy-like changes and OA in mice, do not prove that these genes and proteins play a part in human OA in the general population. However, these models provide insight in the regulation of cartilage homeostasis and can point toward potential essential molecular routes in OA (Table I).

Transcription factors

A number of transcription factors have been identified that regulate chondrocyte differentiation and have an important role in the transition to chondrocyte hypertrophy. Chondrocyte hypertrophy is not under the strict control of a single transcription factor but appears to be regulated by a regulatory network. However, a pecking order of factors is likely, but the exact contribution of different transcription factors to this process has not been elucidated yet. However, it is clear that Runx2 is indispensable for chondrocyte terminal differentiation.

Table I
Regulators of chondrocyte hypertrophy and OA

Hypertrophy regulators	Function/Action	Ref.
Runx2	Master transcription factor in chondrocyte hypertrophic differentiation	11,78,82
HIF2-alpha	Transcription factor in chondrocyte hypertrophic differentiation	
C/EBPbeta	Transcription factor in chondrocyte hypertrophic differentiation	83,84
GADD45beta	Stimulating factor in chondrocyte hypertrophic differentiation, *via* C/EBPbeta	85
Nfat1	Transcription factor in chondrocyte hypertrophic differentiation	86–89
β-catenin	Transcription factor in chondrocyte hypertrophic differentiation	
Smad2/3	Blocks Runx2 function	90,103–106,112,113
Smad1/5/8	Stimulates Runx2 function	90,103–106,112,113
DDR2	Collagen receptor, modulation of Runx2 action	118
Matrilin-3	Extracellular matrix molecule	122
SirT1	Histone deacetylase, transcription regulator	124
TG-2	Extracellular cross-linking enzyme	23,127
Interleukin-8	Cytokine regulating chondrocyte hypertrophic differentiation	108
S100 proteins	Ca-binding proteins	109

Runx2 is a master transcription factor involved in chondrogenic differentiation and completely controls chondrocyte hypertrophic differentiation[11,81]. A core Runx2 regulated element is present in the type X collagen promotor[82]. Runx2 expression is elevated in OA cartilage in humans and experimental models[11,78,82]. What is more, heterozygous RUNX-2-deficient mice show, after surgical induction of knee joint instability, decreased OA severity and reduced type X collagen and MMP13 expression[78].

Hypoxia-inducible factor-2alpha (HIF-2alpha) is a transcription factor that is highly expressed in human OA cartilage and experimental murine OA[83]. HIF-2alpha is important for chondrocyte terminal differentiation. HIF-2alpha, encoded by the gene EPAS1, is a very potent transactivator of COL10A1[84]. It has been shown that HIF-2alpha enhances promoter activities of COL10A1, MMP13 and VEGF through specific binding to hypoxia-responsive elements. Epas1-heterozygous mice showed resistance to OA development and chondrocyte-specific Epas1 transgenic mice demonstrated spontaneous cartilage destruction. These results indicate that HIF-2alpha causes cartilage destruction by stimulating chondrocyte terminal differentiation and up regulation of catabolic enzymes[83,84].

Another transcription factor that has been suggested to be involved in chondrocyte terminal differentiation and OA is CCAAT/enhancer-binding protein beta (C/EBPbeta)[85]. Mouse embryos and isolated chondrocytes with a homozygous deficiency in C/EBPbeta exhibited delayed chondrocyte hypertrophy. In contrast, overexpression of C/EBPbeta in chondrocytes enhanced hypertrophy. Cartilage destruction in instability-induced OA in C/EBPbeta heterozygous was less severe than in wild type mice[85].

Recently, it was reported that C/EBPbeta co-localizes with the regulatory protein Growth arrest and DNA damage-inducible protein 45beta (GADD45beta) in aging articular chondrocytes in senescence-accelerated mice (SAMP1) mice[86]. GADD45beta is a BMP-2 early response gene, *via* the Smad1/Runx2 route, and an essential mediator of MMP13 expression during terminal

chondrocyte differentiation[87–89]. GADD45beta is expressed in early hypertrophic chondrocytes and early OA but not in late stage OA[88].

Nuclear factor of activated T cells 1 (Nfat1) is a transcription factor of a family that has been known to regulate gene transcription in response to T cell receptor-mediated signals in lymphocytes. However, the function of Nfat1 appears not be restricted to lymphocytes, Nfat1 knock out mice show OA development and elevated MMP13 and type X collagen levels at age 3 months and older. Interestingly, in these mice elevated type X collagen expression was associated with increased expression of Ctnnb1, the gene encoding the wnt signaling molecule β-catenin[90].

Transgenic mouse models show that both decreased and elevated levels of β-catenin result in cartilage damage[62,91]. Overexpression of β-catenin led to high expression of hypertrophy markers (e.g., MMP13, type X collagen) by articular chondrocytes while conditional knock down of β-catenin led to chondrocyte apoptosis[62,91]. Both models showed cartilage destruction, but the model showing elevated β-catenin levels and elevated hypertrophy markers demonstrated a pattern of joint destruction more similar to spontaneous OA. Elevated canonical wnt signaling drives OA development by increasing Runx2 activity thereby boosting hypertrophic differentiation of chondrocytes[92–94].

Wnt signaling during chondrocyte hypertrophy is modulated by other extra- and intracellular proteins. It has been shown that TGF-β and BMP signaling is modulated by wnt signaling, most probably by modulation of Smad signaling[95–98]. Moreover, Sox9 and β-catenin interact in their control of chondrocyte differentiation. Sox9 and β-catenin reciprocally influence each others degradation and compete in combination with TCF-LEF with promotor binding sites associated with chondrocyte differentiation[99,100].

The Smads are the most important transcription factors of the TGF-β superfamily, a family that contains amongst other the TGF-β's and the BMPs. Smads play an important role in all aspects of chondrocyte differentiation. These factors control chondrocyte hypertrophy by modulating the function of Runx2. Smads are phosphorylated by TGF-β superfamily type I receptors (activin-like kinases, ALKs). The ALKs determine which Smads are phosphorylated; Smad2 and 3 are phosphorylated by ALK4, 5 or 7 and Smad1, 5 or 8 by ALK1, 2, 3 or 6. In chondrocytes it has been shown that TGF-β can activate both ALK5 and ALK1, activating different response genes, as demonstrated by Finsson *et al.* and our own group[101,102]. Smad2 and 3 are essential signaling molecules in the inhibitory effect of TGF-β on chondrocyte hypertrophy and Smad3 appears to play a more prominent role than Smad2[103]. While terminal differentiation is blocked by signaling *via* Smad2/3, hypertrophy strictly requires Smad1/5/8 signaling[104]. Moreover, in knock out mice it was shown that loss of both Smad1 and 5 results in blocking of chondrocyte hypertrophy. Furthermore, Smad6 and Smurf1 and 2 are known inhibitors of mainly Smad1/5/8 signaling and accelerate proteosomal breakdown of phospho-Smads. Mice overexpressing either Smad6 or Smurf1 demonstrated inhibition of chondrocyte hypertrophy[105]. Smads modulate chondrocyte differentiation by a physical interaction with Runx2. Complex formation of Runx2 with Smad1 is crucial for the function of Runx2 while interaction of Smad3 with Runx2 inhibits Runx2 functioning[106–114]. Apparently, Runx2 is a switch that is either activated or inhibited by different Smads, in this way determining chondrocyte hypertrophy.

We have observed an age-related loss of ALK5 and Smad2/3 phosphorylation in murine articular cartilage and in two experimental OA models, the DMM (meniscus destabilization) model and STR/ORT mice (spontaneous OA)[115]. Expression of non-phosporylated Smad2 or Smad3 was not different in young and old animals. Interestingly, expression of the alternative TGF-β receptor, ALK1, did not diminish to a similar extent as ALK5 during aging and experimental OA[102]. In addition, overexpression of constitutively active ALK1 in chondrocytes resulted in increased expression of MMP13[102]. Moreover, inhibition of ALK5 expression using siRNA resulted in elevated expression of MMP13. These observations indicate that ALK1 signaling, *via* Smad1/5/8, stimulates hypertrophy-like changes in articular chondrocytes.

Non transcription factors

A number of transcription factors of which Runx2 is essential controls chondrocyte hypertrophy. However, other molecules are known to control chondrocyte hypertrophy-like changes. The Discoidin Domain Receptor 2 (DDR2) is a receptor tyrosine kinase that is activated by collagen binding and has been proposed to play a role in OA[116]. Progression of cartilage degeneration in haploinsufficient DDR2 mice with surgically-induced OA was strikingly reduced[117]. Recently it was found that during osteogenic differentiation DDR2 regulates the transactivity of Runx2 by modulating its phosphorylation[118]. DDR2 increased Runx2 transactivation and stimulated type X collagen expression in ADTC5 cells-derived hypertrophic chondrocytes[118]. However, merely over expression of DDR2 in murine cartilage does not lead to cartilage degradation[119]. Only when the pericellular matrix is degraded, for instance by the serine protease HtrA1, DDR2 can be activated by type II collagen binding[119,120]. Expression of HtrA1 in chondrocytes is suggested to be induced by excessive loading, but has also been shown to be associated with chondrocyte terminal differentiation[28,120].

Matrilin-3 is a non-collagenous pericellular matrix protein of which mutations have shown to be involved in early onset OA[121]. Matrilin-3 deficient mice showed in the embryonic growth plate of the developing long bones chondrocytes that prematurely became prehypertrophic and hypertrophic[122]. However, at birth matrilin-3 deficient mice showed no gross skeletal malformations. However, old matrilin-3 knock out mice showed a higher incidence of severe OA. Apparently, matrilin-3 deficiency increases chondrocyte hypertrophy-like changes and leads to a higher incidence of OA.

SirT1 is a histone deacetylase that regulates gene expression, and SirT1 has been implicated as a key factor in aging-related diseases[123]. In human cartilage, SirT1 was hardly detectable in severely degenerated areas while SirRT1 was clearly expressed in less damaged regions[124]. Inhibition of SirT1 by siRNA induced expression of chondrocyte hypertrophy markers, namely a significant up regulation of COL10A1. This is in line with studies showing that SirT1 protects against chondrocyte apoptosis, a hallmark of chondrocyte terminal differentiation[125,126].These observations suggest that SirT1 expression decreases with OA development of OA and that decrease of SirT1 in chondrocytes causes chondrocyte hypertrophy and cartilage degeneration.

TG-2 belongs to a family of protein cross-linking enzymes. Increased TG-2 expression is found in human and experimental OA[127]. In an instability-induced murine OA model it was found that TG-2 deficient mice had reduced cartilage destruction but increased osteophyte formation compared to wild type mice[127]. Chondrocytes from TG-2 knock out mice lost the capacity to undergo hypertrophic differentiation induced by retinoic acid[23]. This indicates that TG-2 knock out mice are protective to OA due to a diminished capacity for chondrocyte hypertrophy-like changes.

Besides these single factors, it becomes more and more clear that inflammatory factors can accelerate chondrocyte hypertrophy. Inflammatory factors such as interleukin-8, ligands for RAGE receptors like the S100 proteins, and NFkB signaling components all appear to play a role in the regulation of chondrocyte terminal differentiation[108–111,114]. A lingering, (sub)clinical inflammatory reaction in the joint might in this way contribute to the unwanted terminal differentiation of articular chondrocytes.

Not only inflammation might modulate chondrocyte terminal differentiation, also mechanical stress, direct or indirect *via* soluble mediators, can stimulate terminal differentiation. A review on mechanics and chondrocyte hypertrophy by Donkelaar and Wilson has recently been published[128].

Apparently, chondrocyte terminal differentiation can be enhanced by a variety of stimuli and regulated intra-cellularly by a number of transcription, and other, factors with a central role for Runx2 (Fig. 1). The default route of chondrocyte differentiation appears to be terminal differentiation, a destiny that is blocked in young and healthy articular cartilage. Inhibition of chondrocyte hypertrophy is a highly regulated process. Primary OA is an age-related disease. Aging is associated with an increase in "chaos", and as a consequence of this related to a loss in regulatory control of biological processes[129–132]. Therefore, it can be postulated that during aging the active inhibition of chondrocyte terminal differentiation is lost and that chondrocytes regain their default differentiation route leading to hypertrophy-like changes. Although an attractive concept, this model is not very easy to prove experimentally.

Chondrocyte hypertrophy-like changes in a conceptual model of human primary, aging-related OA

Human primary OA is an age-dependent, slowly progressive disease. It is clear that several subgroups of primary OA exist in the human population with, until now not fully defined, different characteristics. However, alterations in chondrocyte behavior appear to take a central position in the OA disease process. On the other hand, alternative routes, not chondrocyte-centered, are put forward that might lead to OA.

In OA, different phases can be recognized that are not only different in timing but that can occur simultaneously within a joint, but at different locations (Fig. 2). In this way creating in a particular OA joint a mixture of processes and cellular reactions. It should be clear that the early stages (*initiation* and *progression stage*) will not be diagnosed as clinical OA. Furthermore, one should keep in mind that this conceptual model is only appropriate for primary, age-related OA, and that even for this specific subgroup alternative pathogenic routes are possible.

Initiation stage

A full-grown joint of a young adult starts as a healthy organ with homeostasis of the various tissues in this organ. In the articular chondrocytes of this joint, the step to hypertrophy-like changes is actively blocked, amongst other by TGF-β. During aging, the behavior of the chondrocytes alters and a subset of cells acquires a phenotype that resembles that of hypertrophic chondrocytes, producing MMP13. A striking age-related change in articular cartilage is the loss of ALK5 and Smad2/3 phosphorylation. Phosphorylated Smad3 is a known blocker of chondrocyte hypertrophy. Moreover, HtrA1 a broad-substrate enzyme involved in degradation of the pericellular matrix and expressed in early experimental OA and hypertrophic chondrocytes, is a potent blocker of TGF-β signaling[28,120,133,134]. Which cells acquire an altered behavior, and when, is dependent on a number of variables such as genetic constitution, joint loading, joint architecture, presence of inflammatory stimuli and stochastic processes.

Progression stage

Release and activation of HtrA1, MMP13 and aggrecanases will lead to degradation of the pericellular and intercellular matrix. Focal release of proteoglycans will make collagen more vulnerable to proteolytic degradation. Exposure of the type II collagen matrix will influence chondrocytes by an altered biomechanical environment and changes in chondrocyte differentiation *via* the DDR2 receptor, stimulating hypertrophy-like changes. This process is a self-perpetuating

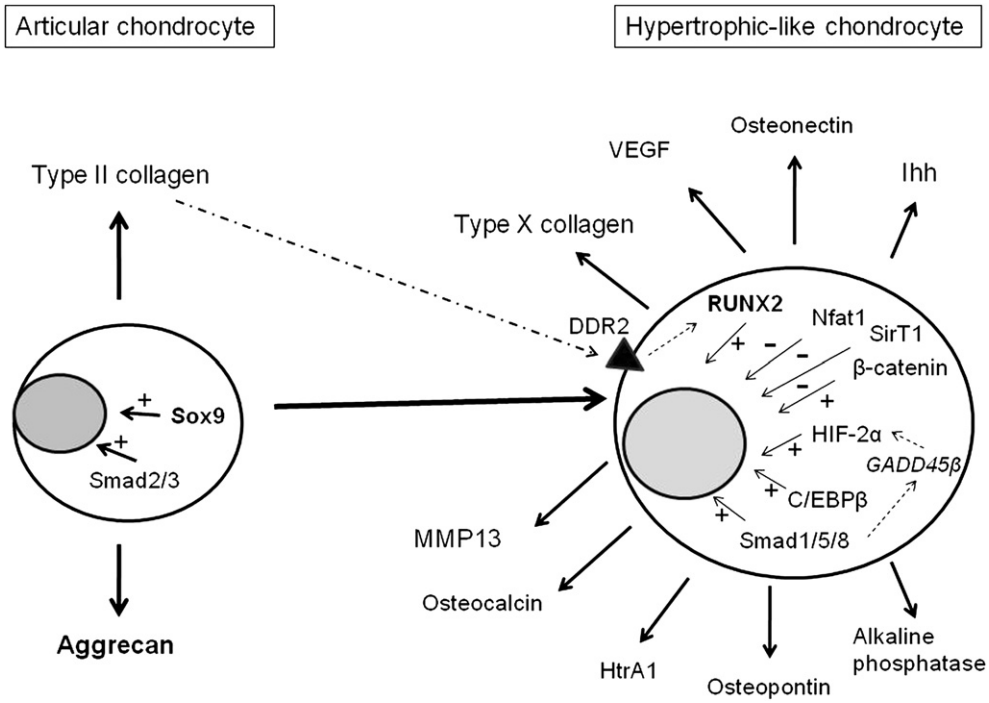

Fig. 1. Factors regulating chondrocyte hypertrophic-like changes and produced by these chondrocytes. Chondrocytes that undergo hypertrophy-like changes produce a number of proteins that are involved in tissue remodeling and calcification. A number of (transcription) factors regulate the differentiation from a normal articular chondrocyte to a terminal differentiated chondrocyte. Factors shown to be involved in OA-related hypertrophy-like changes are shown.

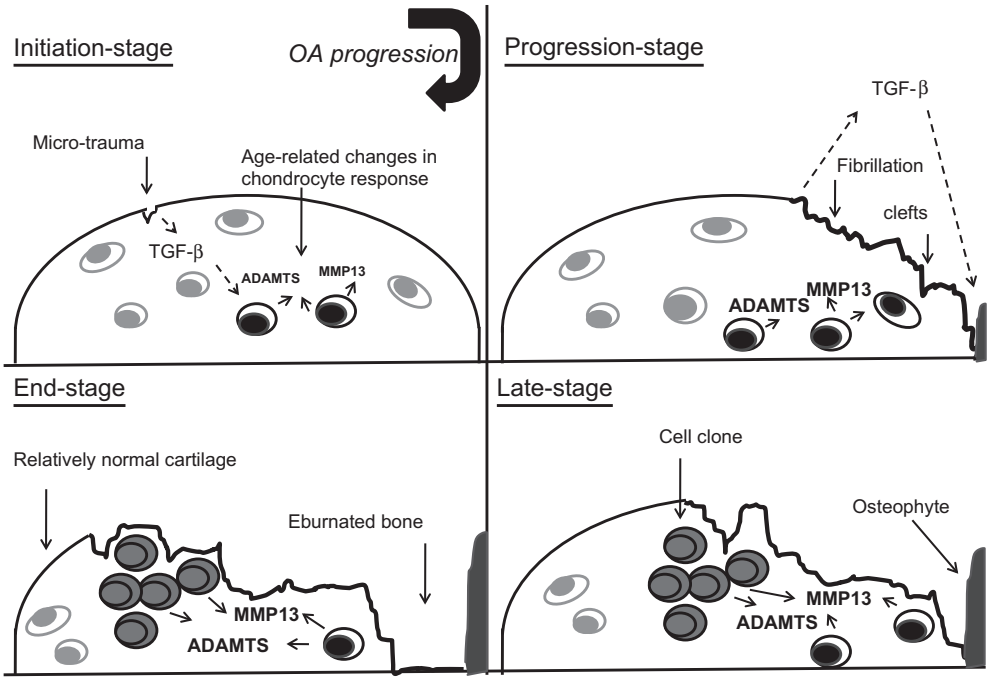

Fig. 2. Chondrocyte hypertrophy-like changes in a conceptual model of human primary OA. Cells that have acquired a "hypertrophy-like" phenotype (**black**) play an essential role in cartilage breakdown by synthesis of MMP13 and ADAMTS4 and 5. TGF-β acts as a chondroprotective factor in young healthy cartilage but looses this action during aging. Chondrocytes in clones neighboring cartilage damage are involved in an unsuccessful attempt at repair, but also contribute to further damage by release of proteolytic enzymes. Of note, besides TGF-β numerous other signaling molecules, enzymes and matrix molecules are involved in the OA disease process.

loop, leading to further damage to the cartilage matrix and driving chondrocyte hypertrophic alterations. In addition, synovial activation, triggered by damage-associated breakdown products, will lead to production of inflammatory cytokines and growth factors, further boosting changes in chondrocyte behavior and subsequent cartilage damage. Factors released from the damaged cartilage matrix and the activated synovial cells will attract inflammatory cells, such as macrophages, and will stimulate synovial fibrosis. Moreover, elevated levels of TGF-β will set off osteophyte formation, originating from the mesenchymal precursor cells in the periosteum.

Late stage

The cartilage contains sites that are heavily damaged. Cartilage neighboring the damaged locations contains a population of chondrocytes that appears to be involved in an attempt to repair the damage. These cells, that show signs of cell proliferation, are located in clusters and show high levels of Smad2/3 and Smad1/5/8 phosphorylation (own data, not shown). The latter is indicative of activation of these cells by members of the TGF-β superfamily. Apparently, different chondrocyte population can be discriminated: chondrocytes that do not show Smad phosphorylation in the less damaged areas of OA cartilage and cells surrounding damage cartilage displaying clear Smad phosphorylation. These cells, although appearing to be involved in an attempt at repair, probably contribute to further cartilage damage by their activated characteristics, exemplified by elevated MMP13 production. It seems that this population of "repair" cells is only present in the highly damaged areas since more intact human cartilage do not show these cell clusters.

End stage

The end-stage will be a mixture of eburnated bone without cartilage, severely damaged cartilage with clefts and fissures, less damaged fibrillated cartilage and cartilage that looks histologically relatively normal. In severely damaged cartilage, chondrocyte clusters are found. At the joint margins, calcified osteophytes are present and the synovial tissue is activated, showing infiltrated inflammatory cells, cell proliferation and fibrosis. Due to the altered biomechanics and release of cytokines and growth factors, the subchondral bone has become sclerotic and shows focal bone cysts. This is the general picture that one gets from end-stage OA cartilage obtained during joint arthroplasty.

The initiation stage is age-dependent and driven by chondrocyte senescence such as changes in chondrocyte receptor expression, maybe because of changes in gene methylation. When the initiation stage is ongoing and irreversible cartilage damage is inflicted (damage to the collagen matrix), the process is a slow but self-perpetuating process mediated by a self-enhancing loop of changes in chondrocyte behavior (hypertrophy-like) and increasing damage to the cartilage matrix. On top of this, neighboring damaged cartilage, chondrocytes are triggered to overcome the damage. However, these cells apparently further contribute to the OA process by the production of MMP13.

Primary OA is a slow, age-related, disease process with a mix of disease stages and cellular responses within a joint. This and the fact that only end-stage human OA cartilage is readily available make reproducible tissue sampling a complicated task. Although most studies report hypertrophy-like changes in OA chondrocytes, the latter could be the explanation for variability between different findings. Alternatively different patient groups exist, with and without hypertrophic changes. Since chondrocyte behavior is not synchronized in an OA joint, and OA starts as a focal process, inhibition of chondrocyte hypertrophy-like alterations could be a therapeutic target to block further OA progression.

Author contributions

PvdK: Literature survey, manuscript preparation.
WvdB: Manuscript preparation.

Conflicts of interest

The authors have no conflicts of interests with regard to the content of this article.

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