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Review

Osteoarthritis year 2010 in review: pathomechanisms

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SUMMARY

Osteoarthritis is characterized by progressive breakdown of articular cartilage. This review summarizes findings of the last year, which shed new light on mechanisms and factors involved in cartilage loss. Evidence is accumulating that the transcription factor hypoxia-inducible factor-2α (HIF-2α) is highly enhanced in OA cartilage and drives catabolic metalloproteinases, including the pivotal MMP-13. In addition, HIF-2α suppresses chondrocyte autophagy, herein promoting chondrocyte apoptosis. The crucial role of MMP-13 is further underlined by reduced OA pathology in MMP-13 deficient mice. An intriguing mechanism to drive MMP-13 production is activation of the chondrocyte discoidin domain receptor (DDR-2) receptor through interaction with denuded collagen type II. The latter might occur in a proteoglycan depleted peri-cellular matrix, where DDR-2 expression is enhanced in OA cartilage and transgenic suppression attenuates experimental OA. The initiating role of ADAMTS-5 in proteoglycan loss appears dependent on interaction with the transmembrane proteoglycan syndecan-4, since syndecan-4 deficient mice are less prone to experimental OA and display reduced ADAMTS-5 activity. Both aging and the osteoarthritis (OA) process itself induce deranged transforming growth factor-β (TGFβ)-receptor expression, causing a shift to dominant usage of the receptor ALK-1, in stead of ALK5 and resulting in a TGFβ mediated catabolic pathway. ALK-1 rather than TGFβ is a promising therapeutic target. Finally, the alarmins S100A8 and 9 have long been considered as markers of inflammatory joint destruction, but now appear to be catabolic mediators.

Hypoxia-inducible factor-2α (HIF-2α) as a novel target

OA is a condition that affects many tissues of the joint. It is now generally accepted that the activated synovial tissue contributes to OA cartilage pathology and the same seems to hold for joint associated fat pads. The infrapatellar fat pad secretes a range of inflammatory mediators including cytokines and adipokines and must be considered as yet another tissue source that influences cartilage metabolism[1].

The present review focuses on recent findings on OA cartilage pathology. Publications from the last half-year of 2009 until September 2010 were searched in Pubmed with attention to mechanisms of OA cartilage destruction and deranged chondrocyte function.

HIF-2α was recently identified as major transcription factor, highly expressed in human osteoarthritis (OA) cartilage and experimental murine OA. HIF-2α, encoded by EPAS1 is strongly induced by NF-kB signaling and as such downstream of well-known mediators like IL-1 and TNF, but also downstream of inflammation and mechanical stress. The group of Chun *et al* reported on a series of elegant target validation experiments[2]. They first showed that ectopic adenoviral overexpression of HIF-2α causes progressive cartilage damage and upregulation of multiple degradative enzymes, including MMP-13. In addition, cartilage destruction is enhanced in transgenic mice with chondrocyte-specific overexpression of HIF-2α. Ultimate proof of a pivotal role was provided by genetic deletion of one allele of HIF-2α and demonstration of suppressed OA pathology in two models of murine OA, induced by destabilized medial meniscus (DMM) or collagenase injection, with concomitant reduction of catabolic factors. In a back to back paper to the above mentioned Nature Medicine study, the group of Kawaguchi identified HIF-2α as an extensive regulator of the endochondral ossification process during OA development[3] and herein broadens the involvement of HIF-2α. It is worthwhile to mention that control of normal homeostasis is not only dependent of HIF-2α, but also relates to the balancing activity of HIF-1α, a factor induced by hypoxia and involved in cartilage formation and maintenance.

With respect to therapeutic targeting of HIF-2α, some caution seems warranted. Most transcription factors are active in multiple

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cell types and to avoid systemic side effects of a putative inhibitor, local targeting of OA affected joints probably is the preferred way forward. In addition, HIF-2α is primarily expressed in early stages of OA, so therapy should be started at recent onset of OA. As an aside, the transcription factor NFAT1 was long recognized to be involved in T cell activation and osteoclastic bone resorption. A recent study[4] identified OA changes in NFAT1 deficient mice, along with enhanced expression of degradative enzymes. Forced expression of NFAT1 with lentiviral vectors rescued normal chondrocyte function and for this transcription factor therapy should be aimed at upregulation.

Deranged autophagy

Autophagy is a process for turnover of intracellular organelles and molecules that protects cells during stress responses. It is now claimed that autophagy is a protective mechanism in normal cartilage and its aging-related loss is linked with cell death and OA.

The group of Martin Lotz evaluated the expression of a set of autophagy related proteins, ULK1, Beclin1 and LC3 in normal and OA human articular cartilage[5]. Expression was strongly reduced in OA chondrocytes, but strikingly high in OA cell clusters. Further analysis in aging mice showed decreased expression of the autophagy markers along with proteoglycan loss and this correlated with an increase of the apoptosis marker PARP p85. A similar pattern was found in surgically induced murine OA.

Intriguingly, an earlier paper of Bohensky *et al*[6] showed that HIF-2α suppresses chondrocyte autophagy, identifying yet another role of HIF-2α in OA cartilage pathology. Of interest, reduction of HIF-2α using siRNA silencing technology enhanced HIF-1α and underlined the balancing role of this set of HIFs.

MMP-13

MMP-13 or collagenase 3 has long been considered as the major enzyme involved in OA cartilage erosion. Suggestive evidence was obtained by transgenic postnatal overexpression of the enzyme in mice, resulting in focal OA cartilage pathology at load bearing sites[7]. Now, ultimate proof of its role is obtained by the demonstration of reduced OA cartilage erosions in MMP-13 deficient mice[8]. OA was surgically induced by DMM and aspects of cartilage pathology were evaluated at 4 and 8 weeks. Erosions were not different between wild type and MMP-13−/− mice at 4 weeks, but at 8 weeks erosions were markedly suppressed in the knockout. Aggrecan loss was already high at 4 weeks in both groups and remained high at 8 weeks. It identifies that MMP-13 deficiency can inhibit cartilage erosion in the presence of aggrecan depletion, and underlines that aggrecan depletion on its own does not drive cartilage erosion. Earlier observations showed that ADAMTS-5 is the pivotal aggrecanase in mice causing aggrecan breakdown in OA[9] and that OA cartilage pathology is reduced in ADAMTS-5 knockout mice. This implies that aggrecan loss paves the way for MMP-13 mediated attack of denuded collagen type II. Both ADAMTS-5 and MMP-13 remain interesting therapeutic targets if side effects on physiology can be limited.

The study in MMP-13 knockout mice further demonstrated that MMP-13 has no critical role in OA associated osteophyte formation. Osteophytes developed undisturbed in the absence of MMP-13. The same holds for chondrocyte hypertrophy as characterized by collagen type X and positive staining for the aggrecan neoepitope DIPEN: it does not in itself lead to cartilage erosion.

Discoidin domain receptor (DDR-2)

Given the problems encountered with side effects of MMP-13 targeting it remains of considerable interest to look for targets upstream of MMP-13. Apart from cytokines and growth factors, DDR-2 is now considered as such a target. DDR-2 is a cell surface receptor tyrosine kinase that preferentially interacts with type II collagen. It is argued that in normal cartilage collagen type II is covered with proteoglycans and does not interact with DDR-2 on the surface of the chondrocyte. However, when proteoglycan depletion occurs, denuded CII is recognized and results in chondrocyte MMP-13 production, along with upregulation of DDR-2 expression which amplifies the process. In an elegant set of *in vivo* studies in DDR-2 deficient mice the relevance of this mechanism for OA has now been demonstrated[10]. Double heterozygous mutant mice were generated, which are both deficient in type XI collagen and DDR-2. The type XI deficiency drives OA cartilage pathology and this was significantly reduced by DDR-2 deficiency, coincident with reduced MMP-13 expression. In addition, the surgical DMM model was induced in the heterozygous DDR-2 mice and impressive reduction of cartilage erosions was found as compared to OA pathology in WT mice. The same group very recently showed acceleration of OA progression by chondrocyte-specific overexpression of DDR-2. The latter condition is also observed in human OA cartilage.

It remains to be explored what local condition really ignites the erosive DDR-2-CII interaction on the chondrocyte surface. It is a long-standing observation in het experimental arthritis field, that cartilage can be heavily depleted of proteoglycans for weeks by sustained local exposure to inflammatory stimuli, but this on its own does not lead to erosions and when inflammation wanes the cartilage proteoglycan content is restored. Apparently, under OA inducing conditions, like CXI deficiency or abnormal load bearing, the peri-cellular matrix around the chondrocyte is further disrupted.

Syndecan-4

Apart from induction of enzymes by activated chondrocytes, their function is further regulated by interaction with matrix molecules and cell surface proteoglycans. Syndecan-4 is a transmembrane heparan sulfate proteoglycan that seems crucial for the activity of ADAMTS-5. Syndecan-4 is specifically induced in type X producing chondrocytes and syndecan-4 immunostaining is abundant in both human and murine OA cartilage. The group of Thomas Pap elegantly showed that the loss of Syndecan-4 activity markedly reduced OA cartilage pathology in the murine DMM OA model. This was demonstrated both in Syndecan-4 knockouts as well as in WT mice, locally treated by intraarticular injections with Syndecan-4 specific antibodies[11]. *In vitro* studies identified direct interaction of Syndecan-4 with ADAMTS-5. In addition, it is claimed that ADAMTS-5 activity is dependent on MMP-3 and the latter activity is controlled by Syndecan-4.

Potentially, the story is more complex. Matsui *et al* demonstrated accelerated development of aging associated and instability induced OA in osteopontin-deficient mice, characterized by increased MMP-13 activity[12]. It identifies a protective role of osteopontin. Osteopontin is a noncollagenous matrix protein, originally described in bone but later on also found in hypertrophic cartilage. It contains an RGD sequence that interacts with integrin receptors, and its function is negatively regulated by Syndecan-4[13]. So, osteopontin has a protective role in OA, which is suppressed by Syndecan-4. Syndecan-4 deficiency will unmask osteopontin activity and this might contribute to the observed reduction of OA in Syndecan-4 knockouts.

Transforming growth factor-β (TGFβ)

TGFβ is considered as a crucial anabolic growth factor for cartilage and is heavily used as a driver of *in vitro* cartilage

engineering. However, the molecule also has a dark side, it can cause OA like tissue pathology upon sustained exposure in the joint and it is a pivotal driving force for osteophyte formation[14,15]. This dualistic role has long remained enigmatic, but recent insight in receptor usage has provided further clues. The protective role of TGFβ runs through the ALK5 receptor and the downstream signaling pathway SMAD2/3, with induction of TIMP, counteraction of IL-1 and prevention of hypertrophy. In contrast, when TGFβ interacts with the ALK-1 receptor, it signals through SMAD 1,5,8 and induces degradative enzymes (MMP-13). We recently demonstrated that there is a shift in receptor usage with aging and during OA, from the protective ALK5 toward the destructive ALK-1[16], implying a dominant pathogenic role of TGFβ. It makes ALK-1 a better therapeutic target as compared to TGFβ.

The role of the protective SMAD3 pathway is further underlined by the demonstration of aberrant hypertrophy in SMAD3-deficient murine chondrocytes, which can be rescued by TGFβ – activated kinase (TAK-1)/activating transcription factor 2 (ATF-2) signaling and restoration of p38 activation[17]. Furthermore, genetic variation in the SMAD3 gene was found to be associated with human hip and knee OA[18].

S100A8/A9

The alarmins S100A8 and A9 have long been considered as markers of destructive processes in the joint. Previously known as MRP8 and MRP9 (myeloid related protein) these factors were found at high levels in synovial fluid, reflect leucocyte activity and coincide with progression of joint damage in rheumatoid arthritis. Now it becomes clear that these alarmins are also mediators of inflammation and can signal through TLR4[19]. We have shown that cartilage destruction in experimental arthritis is reduced when the model is induced in S100A8/9 deficient mice[20]. Furthermore, expression of these proteins was markedly enhanced in arthritic cartilage, and *in vitro* S100A8 shows a direct potency to stimulate chondrocytes to produce MMPs and to cause aggrecanase mediated peri-cellular matrix degradation[21]. A recent study of Zreiqat *et al* more extensively explored dose dependent upregulation of various ADAMTSs and MMPs[22] and provided evidence for expression of S100A8 and A9 in cartilage of early but not late experimental OA. We recently evaluated the impact of S100A8/9 deficiency on the occurrence of experimental OA. We did not see a phenotype in the DMM model, but found clear suppression of cartilage pathology in the collagenase induced instability OA. The latter model is characterized by considerable synovial activation and apparently only under those conditions synovial S100A8/9 make a significant contribution. The same might hold true in humans, with an expected role in OA patients with an inflammatory phenotype.

Final remarks

This review focused on some major developments in 2010. One pathway that deserves some attention is the Wnt/β-catenin signaling, although no major breakthroughs were reported in the period covered by this paper. Earlier work showed enhanced β-catenin expression in human OA cartilage and elegant studies in transgenic mice identified that conditional, cartilage specific activation in adult mice induces an OA like phenotype[23]. Some recent reviews are suggested for further reading[24,25].

Figure 1 summarizes major elements of the studies discussed above. It shows multiple pathways of chondrocyte triggering and downstream transcription factors, with a final common pathway of MMP-13 mediated collagen type II breakdown and ultimate cartilage erosion. Fine-tuning of ADAMTS-5 activity is under the control of Syndecan-4 and Osteopontin, with an intermediate role

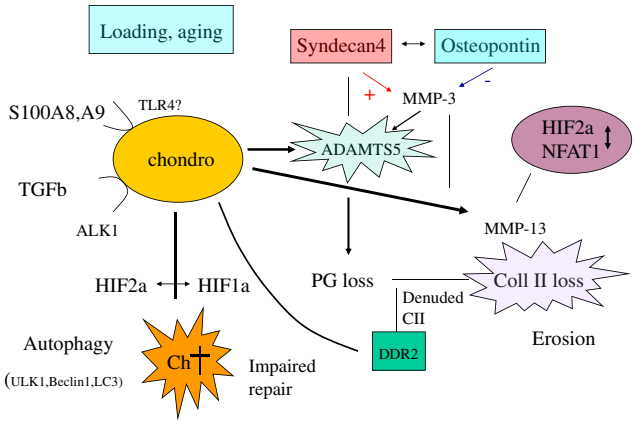

Fig. 1. Pathways of OA cartilage pathology.

of MMP-3. The latter is also crucial in MMP-13 activation. Deranged autophagy causes cell death and impairs regenerative capacity of the cartilage.

Conflict of interest

I herein declare that I have no conflict of interest

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