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Age-related alterations in TGF beta signaling as a causal factor of cartilage degeneration in osteoarthritis

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Abstract.
BACKGROUND: Age is the most important risk factor for primary osteoarthritis (OA). Members of the TGF-β superfamily play a crucial role in chondrocyte differentiation and maintenance of healthy articular cartilage.
OBJECTIVE: We have investigated whether age-related changes in TGF-β superfamily signaling components play a role in the relationship between OA-related cartilage degradation and aging.
MATERIAL AND METHODS: The relationship between age, OA and TGF-β superfamily signaling was studied using murine experimental OA models, aging mice, bovine articular cartilage and human OA cartilage. The effects of TGF-β on cartilage homeostasis was studied with immunohistochemistry, Q-RT-PCR and signaling pathway analysis with Western blotting and the application of specific TGF-β inhibitors.
RESULTS: We have found that TGF-β loses its protective effects in old cartilage. Moreover, we found that on chondrocytes, TGF-β not only signals via the canonical type I receptor ALK5 (TGFBR1) but also via the ALK1 (ACVRL1) receptor. Remarkably, signaling via ALK5 (Smad2/3 route) results in protective while ALK1 signaling (Smad1/5/8 route) results in deleterious responses in articular chondrocytes. In cartilage of aging mice it was detected that the ALK1/ALK5 ratio is significantly increased, favoring TGF-β signaling via the Smad1/5/8 route, inducing changes in chondrocyte differentiation and matrix metalloproteinase-13 (MMP-13) expression. Moreover, human OA cartilage showed a significant correlation between ALK1 and MMP-13 expression. Since in mice aging and OA in often goes hand in hand, we also analyzed age-related expression of TGF-β superfamily related signaling molecules in healthy bovine cartilage in an age range from 6 months to 14 years. In this cohort of aging cartilage, we found that mainly signaling receptors determining the Smad2/3 pathway were decreased with age while Smad1/5/8-related signaling molecules did not alter, confirming our findings in aging mice.
CONCLUSIONS: Old cartilage appears to be less protected by TGF-β and shows significant alterations in TGF-β signaling pathways. Loss of the protective Smad2/3 pathway during aging can provide an explanation for the relationship between OA and aging.

Keywords: Osteoarthritis, aging, TGF-β, ALKs, BMPs

1. Osteoarthritis and aging

The only way to stop ageing is passing away early. In contrast to the early days of our human history, life expectancy has increased, and is still increasing significantly. Old age is a risk factor for many diseases but especially for primary osteoarthritis (OA), aging being the risk factor most strongly related to OA. Osteoarthritis is the most common joint disease and is characterized by pain and a loss of joint

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function. Osteoarthritis is a whole joint disease involving all tissues in the joint but its main pathophysiological phenomenon is cartilage damage. Based on our initial findings in the early nineties of the last century we have focused on the role of TGF-β in cartilage physiology and especially on the changing role of TGF-β in cartilage homeostasis during aging.

2. TGF-β and isolated chondrocytes

Our initial interest in the role of TGF-β in cartilage homeostasis was raised by two papers of Seyedin et al. [1]. In these papers a factor was described, isolated from demineralized bone, that was called cartilage-inducing factor-A (CIF-A) that appeared to have a 100% similarity with TGF-β. Furthermore, CIF-A and TGF-β stimulated the synthesis of cartilage-specific macromolecules in rat muscle mesenchymal cells. This observation stimulated our interest to study TGF-β effects on isolated bovine chondrocytes and intact murine cartilage [2,3]. In these early studies we showed that the effect of TGF-β on bovine chondrocytes was dependent on the period in culture of these chondrocytes. TGF-β had opposite effects with regard to DNA synthesis and proteoglycan synthesis in freshly isolated chondrocytes and chondrocytes cultured for one week. These early observations showed us that TGF-β effects are not always straight forward, and we speculated that these differential responses might be related to differences in TGF-β receptor expression. Unfortunately, in those days little was known about TGF-β signaling.

Expression of TGF-β receptors on chondrocytes was studied with radioactive TGF-β in combination with affinity labeling and SDS-PAGE. We could demonstrate a size difference in the so-called type II receptor between freshly isolated and cultured chondrocytes [4]. The type II TGF-β receptor of cultured chondrocytes appeared to be about 15 kilodaltons smaller than the type II TGF-beta receptor expressed on freshly isolated chondrocytes. Some years later an isoform of the TGF-β receptor (RII2), containing an insertion of 25 amino acids in the extracellular domain of the receptor, was identified by PCR [5]. We found that this RII receptor was not expressed in bovine articular chondrocytes and no changes in expression were detected comparing young and old murine articular cartilage and during cartilage repair after mild proteoglycan depletion [6]. This indicated that it was unlikely that age-related TGF-β responses and differential TGF-beta responses of chondrocytes are related to differences in the relative expression of the two TGF-β type II receptor isoforms. Around the same time it became clear that TGF-β signaled via a complex of type I and type II receptors. The type I receptors, called activin receptor-like kinases (ALKs), act downstream of the type II receptor. The ALKs determine receptor specificity and the particular activated intracellular pathway. TGF-β was shown to signal via the type I receptor ALK5 (TGF-βRI) and activate the intracellular Smad2/3 route [7].

3. TGF-β counteracts IL-1 effects in murine knee joints only in young animals

Also in the early nineties of the last century we started with our *in vivo* studies of TGF-β in murine knee joints. We could show that a single intra-articular TGF-β injection into 3 months old C57Bl/6 mice resulted in elevated proteoglycan synthesis in the articular cartilage and that TGF-β was able to counteract the inhibiting effects of interleukin-1 (IL-1) on proteoglycan synthesis [8]. In later studies, we showed that blocking endogenous TGF-β, using the specific TGF-β inhibitor latency-associated peptide (LAP), led to more profound inhibition of proteoglycan synthesis by IL-1 and also blocked the repair response that is observed in a later stage after IL-1 injection [9]. Moreover, over expression of

TGF-β using an adenoviral vector, also counteracted IL-1 effects and even stimulated cartilage repair [9]. Furthermore, blocking TGF-β activity using a soluble TGF-beta-RII receptor, that scavenges TGF-β, led to increased cartilage damage in a model of experimental osteoarthritis [10]. These results strongly indicated that TGF-β is a powerful blocker of IL-1-induced cartilage damage and that TGF-β stimulates cartilage repair, at least in young mice.

Our studies clearly showed that TGF-β is a highly cartilage protective factor in young animals but that this protective capacity was lost in old animals [9,11]. In mice of 1,5 year old and older, TGF-β was unable to counteract the deleterious effects of IL-1 on articular cartilage. An important role for endogenously released TGF-β as a cartilage protective factor and a loss of this action in old animals is in line with our earlier observations in which we compared IL-1 effects in old and young mice. In old murine articular cartilage, IL-1-induced suppression of proteoglycan synthesis is more prolonged and the amount of time needed to restore the cartilage matrix is significantly longer than in young animals [12]. The difference in the protective effects of TGF-β between young and old animals raised the interesting question about the mechanism of this striking difference.

4. Loss of TGF-β signaling in cartilage of old mice

To elucidate the mechanism behind the loss of TGF-β-related chondroprotection we compared the expression of TGF-β ligands and intracellular signaling molecules in the cartilage of young and old mice. We analyzed expression of TGF-β 1, -2 and -3, TGF-β RI (ALK5), TGF-β RII, Smad2, -3, the co-Smad (Smad4), and the inhibitory Smads (6 and -7) and phosphorylated Smad-2/3. Our experiments revealed that mainly expression of the TGF-β receptors decreased with age [9]. Although the expression of the Smad proteins itself was not altered, also the number of cells expressing phosphorylated Smad2/3 showed a strong drop in old mice. We concluded that the inadequate chondroprotective effect of TGF-β in old mice is not due to a decreased level of intracellular signaling molecules or an up regulation of intracellular inhibitors, but is due to an intrinsic reduction in TGF-β receptor expression and a subsequent loss of Smad2/3 signaling.

During the same period it became clear that ALK5 was not the only TGF-β type I receptor, but that at least in endothelial cells TGF-β also signaled via ALK1, resulting in totally different effects on these cells [13,14]. An observation reminding us of our initial ideas with regard to the differential effects of TGF-β on chondrocytes (see above). Studies by us and the group of Philip showed that also in chondrocytes, TGF-β signals, like in endothelial cells, via both ALK5 and ALK1 and activates respectively the Smad2/3 route and the Smad1/5/8 route [15,16]. We found that transfection of chondrocytes with constitutive active ALK1 increased MMP13 expression, an enzyme essential for type II collagen degradation and a marker for OA chondrocytes. In contrast, inhibiting ALK1 expression using siRNA decreased MMP13 expression [16]. Blocking of ALK5 by siRNA resulted in increased MMP-13 expression. Moreover, in human OA cartilage, ALK1 expression was highly correlated with MMP-13 expression, whereas ALK5 expression correlated with aggrecan and collagen type II expression [16]. This indicates that ALK5 signaling has a protective effect while ALK1 signaling can have a deleterious effect on articular cartilage.

Furthermore, in cartilage of mouse models for aging and experimental OA, ALK5 expression was decreased far more strongly than ALK1 expression. This resulted in an increased ALK1/ALK5 ratio in old and OA cartilage. This was associated with corresponding changes in the respective downstream markers of ALK1 (inhibitor of DNA binding-1, Id1) and ALK5 (plasminogen activator inhibitor-1, PAI-1) [16].

This has led to our strong belief that in young cartilage TGF-β is a highly protective protein which function is lost in old cartilage. In old cartilage TGF-β even can have a deleterious effects by signaling via ALK1, up regulating MMP13 expression and chondrocyte terminal differentiation.

In mice and man old age and OA go hand in hand. To determine whether a decrease in the Smad2/3 signaling pathway really precedes cartilage damage we used healthy bovine cartilage of an age range between 6 months and 14 years. We could show that also in intact bovine cartilage Smad2/3 signaling was reduced significantly during aging while Smad1/5/8 signaling did not decrease notably (unpublished). These findings indicate that loss of protective TGF-β signaling appears to be a generalized finding and that loss of protective TGF-β signaling precedes cartilage damage.

5. Relevance of ALK1 and ALK5 signaling for OA development

Loss of TGF-β signaling is related to OA development in man and mice. A mutation in the asporin gene that leads to increased inhibition of TGF-β is associated with early OA development in Asian and Greek populations [17–19]. Moreover, pointing to an important role for Smad3 in cartilage homeostasis and OA development is the observation that loss of function mutations in Smad3 result in early OA onset in man [20,21]. Earlier it had been found in Smad3 knock out mice that Smad3 signals inhibit terminal hypertrophic differentiation of chondrocytes and that Smad3 is essential for maintaining articular cartilage [22]. This conclusion is supported by the observation that accelerated degradation of Smad3 is associated with increased MMP13 expression and cartilage degeneration in mice [23].

The effect of Smad3 on cartilage can be two-headed. Signaling will inhibit the action of inflammatory cytokines, like IL-1 [24], and will decrease the production of proteases and increase the synthesis of protease inhibitors [25,26]. In addition, signaling via the Smad2/3 route will inhibit chondrocyte terminal differentiation while signaling by the Smad1/5/8 pathway will stimulate this [22,27–29]. Both direct inhibition of cartilage damage and inhibition of chondrocyte terminal differentiation by Smad2/3 signaling is crucial for homeostasis of articular cartilage. Moreover, signaling via Smad1/5/8 will push chondrocyte to an OA-like (hypertrophic) phenotype.

6. Concluding remarks

TGF-β signaling via Smad2/3 is essential for maintenance of normal, healthy cartilage. Loss of this signaling pathway will release an important break on cartilage damage inducing factors, such a trauma and inflammation, and this loss will make terminal differentiation of chondrocytes possible. The latter can be even accelerated via remaining Smad1/5/8 signaling. Once cartilage is damaged the role of TGF-β will change again. Elevated levels of TGF-β will stimulate mesenchymal stem cells to undergo chondrogenesis (osteophyte formation) and will stimulate fibroblasts to proliferate and deposit matrix (synovial fibrosis). Furthermore, TGF-β will stimulate efforts to repair cartilage by residing chondrocytes and invading cells at sites with severe cartilage damage. So, from a physiological protective role of TGF-β in young cartilage, loosing this function during aging, TGF-β will change in a factor that mediates a number pathological aspect of the OA joint (osteophytes, fibrosis) but remains a factor stimulating attempted cartilage repair by stimulation of local and invading stem cells to make new cartilage (Fig. 1).

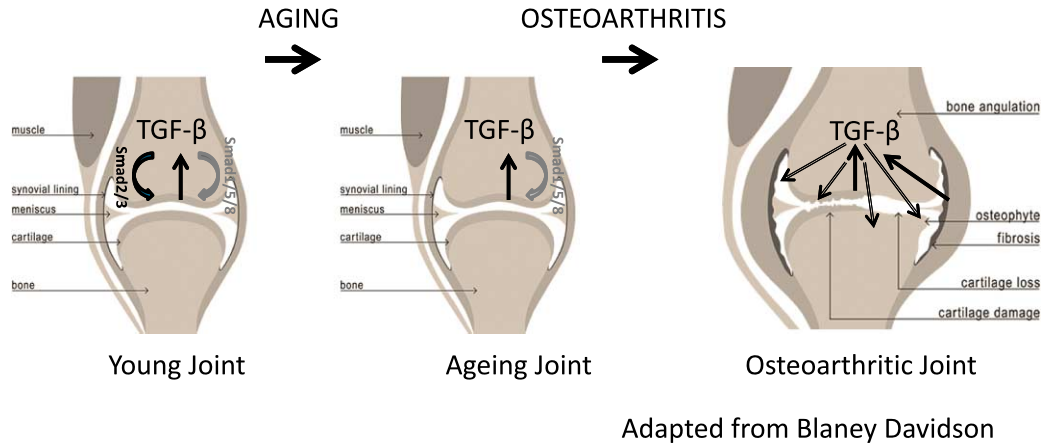

Fig. 1. Changes in the role of TGF-β during ageing and OA. In a young joint TGF-β is a protective factor that blocks the action of inflammatory cytokines and chondrocyte terminal differentiation. During aging this protective effect of TGF-β is lost and TGF-β can even stimulate chondrocyte hypertrophy. In an OA joint TGF-β is involved in numerous processes such osteophyte formation, synovial fibrosis and the stimulation of cartilage repair (chondrogenesis) by invading and resident cells (Modified from PhD thesis of E. Blaney Davidson, 2007). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BME-140976.)

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