JBM J Bone Metab 2018;25:65-72
https://doi.org/10.11005/jbm.2018.25.2.65
pISSN 2287-6375 eISSN 2287-7029

Review Article

Differential Role of Transforming Growth Factor-beta in an Osteoarthritic or a Healthy Joint

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Received: January 3, 2018
Accepted: May 26, 2018

No potential conflict of interest relevant to this article was reported.

Transforming growth factor-β (TGF-β) is a cytokine that plays an important role in both normal joints and joints affected by osteoarthritis (OA), the most common joint disease. However, the role of this pleiotropic cytokine in a normal healthy joint is very different from its role in an OA joint. In a normal synovial joint, active TGF-β is only present after joint loading and only for a short period. In contrast, permanent and high levels of active TGF-β are detected in OA joints. Due to this difference in levels and exposure period of joint cells to active TGF-β, the function of TGF-β is strikingly different in normal and OA joints. The consequences of this difference in TGF-β levels on joint homeostasis and pathological changes in OA joints are discussed in this review.

Key Words: Cytokines, Osteoarthritis, Transforming growth factor beta

OSTEOARTHRITIS (OA)

OA is the most common joint disease with an increasing prevalence due the ageing population of the Western world and until now not fully identified factors related to our Western society lifestyle.[1] OA is characterized by loss of articular cartilage, osteophyte formation, subchondral bone changes, fibrosis and in many patients inflammation. The major clinical symptoms are joint pain, stiffness and loss of function. In general, OA is progressive and no widely applied effective therapy is available. The ultimate solution for an end stage OA joint is replacement of the damaged joint by an artificial one. In about twenty percent of the patients this procedure does not result in a pain free joint. The homeostasis of a healthy joint is dependent on the proper interaction between joint cells and tissues. Disturbed cellular communication will result in loss of tissue integrity and damage to all joint tissues. In this way OA can be considered as a whole joint disease in which joint homeostasis is lost and in the joint has entered an irreversible disease state.

TRANSFORMING GROWTH FACTOR-β (TGF-β)

TGF-β is a member of a protein family of over 35 members that can be found in all multicellular organisms.[2] TGF-βs regulate many important processes in both health and disease, including cell proliferation, tissue formation, and repair and inflammation. In mammalian tissues three peptides are identified TGF-β1, TGF-β2,

and TGF-β3. These peptides have a high degree of homology but differ in their tissue specific expression. The function of the three different peptides in development and physiology can be partly drawn from knock out studies in mice. Knock out of TGF-β1 is in many cases lethal as a result of defective haematopoiesis and endothelial differentiation during embryogenesis.[3] Mice deficient for TGF-β1 that are born alive develop a rapid wasting syndrome and massive inflammation resembling autoimmune disorders.[4,5] Deficiency for TGF- β2 leads to multitude of developmental defects including, craniofacial, limb, spinal column, eye, lung, inner ear and urogenital abnormalities.[6] TGF-β3 also plays a role in development since homozygous TGF-β3 knockout mice have a cleft palate.[7] There is no phenotypic overlap between TGF-β1, TGF-β2, and TGF-β3 knockout mice indicating numerous but non-overlapping functions between the TGF-β isoforms.

1. TGF-β signaling

TGF-β's are synthesized as large pro-proteins that have to be activated before receptor binding and signal via a heteromeric complex of transmembrane serine/threonine receptors, so-called type I and type II receptors, and accessory proteins.[8] The type I receptors, also called- activin receptor-like kinases (ALKs), are activated by the type II receptors and intracellular signaling is initiated by C- terminal phosphorylation of receptor-regulated (R)-Smad proteins by the type I receptors.[9] The canonical TGF-β type I receptor is ALK5 but also other ALKs have been shown to be able to propagate the TGF-β signal. In chondrocytes ALK1 has been identified as a TGF-β receptor.[10,11] Activation of ALK5 stimulates phosphorylation of Smad2 and Smad3 while ALK1 activates the Smad1/5/8 pathway.[10,11] Phosphorylated R-Smads form heterotrimers with Smad4 and this complex is transported to the nucleus. In the nucleus this complex, in association with enhancers and suppressors, regulates gene expression. Activation of the Smad2/3 and Smad1/5/8 pathway has differential effect on gene expression and regularly antagonize each other.

In many cell types it has been shown that the TGF-β concentration to which the cells are exposed determines the preferred-signaling route. In primary human fibroblasts low TGF-β concentrations (≤1 ng/mL) activate the Smad2/3 route while high concentrations (>5 ng/mL) mainly stimulate the Smad1/5/8 pathway.[12] A similar pattern was found in myofibroblast cell lines and endothelial cells.[13-15] This is most likely a result of the different affinities of TGF-β for different receptor complexes. However, how cellular signaling is determined by the active TGF-β concentration will depend on the absolute and relative number of different TGF-β receptors on the cell membrane of different cell types. A consequence of this is that exposure of cells to different TGF-β contractions will results in differential regulation of gene expression at low or high TGF-β concentrations.

2. TGF-β levels in healthy and OA joints

Healthy human cartilage contains a large pool TGF-β that is normally not readily accessible for the chondrocytes.[16] The predominant form of TGF-β in articular cartilage appears to be TGF-β1 (60%-85% of total) and high quantities of total TGF-β1 have been detected in articular cartilage (68.5±20.6 ng/mL).[17] The first measurement of TGF-β activity in joint fluids of patients with joint diseases, using bioassays, has been published by Fava et al.[18]. They found high levels of active TGF-β in synovial fluid of rheumatoid arthritis patients (10 ng/mL), OA patients (4 ng/mL) and gout patients (8 ng/mL) but no significant TGF-β activity in synovial fluid of patients with avascular necrosis. Acid activation resulted in a 3 to 4 –fold increase in TGF-β activity. Furthermore, the synovial fluid of normal temporomandibular joints does not contain detectable levels of active TGF-β but elevated levels are measured in patients with temporomandibular OA.[19] Moreover, there is evidence that TGF-β is proteolytically activated in synovial fluid and elevated levels of proteases are present in OA synovial fluid.[20] These results clearly indicate that active TGF-β levels will be very low or absent in normal articular joints and elevated in joint diseases such as OA.

It can be concluded that cells in normal joints are not exposed to high TGF-β levels but that this will change once OA develops. The elevated levels of TGF-β in OA joints will activate cells that are normally not exposed to high levels of active TGF-β and this will result in altered cellular differentiation and can contribute to pathogenesis if these high TGF-β levels are enduring.

Loading temporarily activates TGF-β in articular cartilage

TGF-β is stored in high amounts in the articular cartilage matrix but in an inactive form as part of a large latent com-

https://doi.org/10.11005/jbm.2018.25.2.65

plex. It has been reported that mechanical loading can activate TGF-β. In a stiff matrix, like articular cartilage, mechanical force, in collaboration with presumably integrins, is able to release mature TGF-β from its latency-binding peptide (LAP) that confers inhibition of receptor binding of TGF-β.[21,22] Articular cartilage is a tissue that is regularly loaded and its main function are to withstand and propagate mechanical forces. On top of the load resisting function of cartilage it has been shown that regular loading is essential to keep articular cartilage vital. Spinal cord injury patients, that do not load their cartilage recurrently, lose their cartilage at a faster pace than OA patients.[23]

Not only load is necessary to keep cartilage healthy, but also active TGF-β signaling. Chondrocyte specific loss of Smad3, ALK5 or the TGF type II receptor in mice all result in OA development at an early age.[24-27] Supporting the findings in mice is the observation that also in humans defective Smad3 results in OA at a young age.[28] These data indicate that to keep cartilage healthy both regular loading and active TGF-β signaling, via Smad3, is needed.

We showed that bovine articular chondrocytes show active TGF-β signaling immediately after death but that active TGF-β signaling is already decreasing within 2 hr in the absence of loading.[29] Compression, at a physiological level (3 MPa, 1 Hz, 30 min), rapidly restored TGF-β signaling. Expression of TGF-β response genes was significantly reduced 24 hr after loading and also this response could be rapidly reactivated by loading. The role of TGF-β in this process, and in particularALK5, could be shown by the fact that load-induced effects could be blocked by a specific ALK5 inhibitor.[29] Furthermore, the effects of loading could be fully mimicked by exogenous addition of active TGF-β but not by activin or bone morphogenetic proteins (BMP).[29]

These observation indicate that active TGF-β signaling is the default mode of cartilage when regularly loaded. We could show that the main function of TGF-β activation was prevention of hypertrophic differentiation of articular chondrocytes. Increased expression of type X collagen, a hypertrophy marker, in the absence of loading was blocked by either addition of exogenous TGF-β or loading. Moreover, loading-induced TGF-β signaling also up regulated the expression of ALK5 on chondrocytes while down regulating the expression of ALK1. Also expression of TGF-β1, in its latent form, was stimulated by loading. The elucidated mechanism shows that loading-induced TGF-β signaling signaling is an elegant system that prevents chondrocyte hypertrophy and keeps articular cartilage healthy.

3. Effect of elevated active TGF-β levels in OA joints

Cells in joint tissues that are normally not exposed to active TGF-β are exposed to high levels in OA joints. Even in articular cartilage a situation of temporarily activation of TGF-β by loading, but fast inactivation of activated TGF-β by the cartilage matrix,[30] is altered to a state with a continuous exposure to elevated active TGF-β levels. Since high levels of TGF-β activate different receptor pathways than low levels, and continuous exposure will also alter chondrocyte activation, the role of TGF-β on cartilage will be different in an OA joint than in a normal joint. It can be expected that preferentially the Smad1/5/8 pathway will be activated (via ALK1), a pathway that is not activated in intact healthy cartilage by TGF-β (unpublished results). The changed situation of high TGF-β levels will drive chondrocytes preferentially in the direction of hypertrophy since it has been shown that the Smad1/5/8 pathway stimulates this in chondrocytes.[31-34] Due to the altered TGF-β concentrations to which the chondrocytes are exposed the function of TGF-β changes from a factor that blocks chondrocyte hypertrophy (Smad2/3 signaling) to a factor that facilitates chondrocyte hypertrophy (Smad1/5/8 pathway). As a consequence the protective effect of TGF-β on articular cartilage will be lost. We and others have found a switch from Smad2/3 to Smad1/5/8 signaling in age-related OA models which appears to confirm the altered role of TGF-β in OA cartilage compared to normal cartilage.[11,35]

4. Changes in subchondral bone

Subchondral bone is one of the tissues that shows alterations in OA joints. Accelerated remodeling, indicated by cyst formation and subchondral sclerosis are hallmarks of OA joints.[36-38] Several studies indicate that TGF-β plays a role in the alterations in the subchondral bone in OA. A correlation between total TGF-β and OA severity has been reported for human hip OA and increased mRNA of TGF-β1 and TGF-β3 subtypes has been shown in osteoblasts in subchondral bone from knee joints with OA.[39,40] Rats with experimental OA (anterior cruciate ligament transection [ACLT] model) treated with alendronate showed inhi-

bition of subchondral bone resorption, vascular invasion and the local release of active TGF-β.[41] Activation of TGF-β has been shown in a mouse model of OA (ACLT model).[42] These high active TGF-β concentrations induced the formation of nestin-positive mesenchymal stem cell clusters in subchondral bone. Furthermore, elevated expression of TGF-β1 in osteoblast induced OA while TGF-β inhibition resulted in decreased development of OA in mice with experimental OA.[42] Treatment of mice and rats with posttraumatic OA (ACLT model) with halofuginone, a TGF-β inhibitor, attenuated subchondral bone deterioration.[43] Moreover, in a guinea pig model of spontaneous OA changes in subchondral bone were in parallel with active TGF-β expression. These data indicate that high active levels of TGF-β contribute to the pathogenetic changes observed in subchondral bone in OA joints.

5. Osteophyte formation

In OA not only bone remodeling takes place but also the formation of new bone as a result of chondrogenesis and ostechondral ossification, osteophyte formation. Osteophytes are a typical characteristic of OA and are not formed in joints affected by rheumatoid arthritis. Osteophytes are bony outgrowths that originate from progenitor cells being located in the periosteum at the border line of the cartilage and bone.[44] Bolus injections of TGF-β1 or adenoviral overexpression of TGF-β1 in mouse knee joints induce the formation of osteophytes with structure and localisation similar to the osteophytes found in spontaneous murine OA.[45,46] Injection BMP's also results in osteophyte formation but these osteophytes appear to mainly originate from the growth plate chondrocytes.[47,48]

Inhibition of TGF-β activity, either blocking receptor binding by the use of the soluble type II receptor or blocking intracellular signaling using Smad7, significantly decreased osteophyte formation in experimental OA in mice.[49,50] Mice with drug-inducible chondrocyte-specific overexpression of BMP-2 did not show more osteophyte formation than control mice.[51] However, osteophyte formation was greatly boosted in BMP-2 overexpressing mice when OA (destabilization of medial meniscus model) was induced in these mice. When under these conditions TGF-β1 activity was inhibited also the formation of osteophytes was strongly reduced (unpublished). These results indicate, that at least in experimental models, TGF-β is the main driver of osteophyte formation but that this process can be enhanced by BMP activity. Elevated levels of active TGF-β, absent in normal joints, activate progenitor cells residing in joint tissues, such as periosteum, to undergo chondrogenesis and make new bone. This is confirmed by *in vitro* studies which confirm that TGF-β is the most potent cytokine to induce chondrogenesis in progenitor cells.[52,53]

6. Synovial fibrosis in OA joints

Patients with OA complain of pain but also in many cases of joint stiffness.[54,55] Joint stiffness can be a result of fluid accumulation in the affected joint but also of fibrosis of the joints capsule. Fibrosis can be considered as a exaggerated repair process of damage tissues. TGF-β is known to be the most potent profibrotic factor. Either injection or adenoviral expression of active TGF-β result in synovial fibrosis in mouse knee joints.[47,56] Remarkably, fibrosis is more pronounced after adenoviral overexpression of TGF-β, prolonged exposure to moderate levels, while osteophyte formation is more pronounced after TGF-β injection, short exposure to high concentrations. This suggest that different cells types are activated by different regimes of TGF-β exposure, progenitor cells move forward into the direction of chondrogenesis by peak levels of TGF-β and fibroblasts are induced to proliferate and synthesize matrix molecules by prolonged elevated concentrations of TGF-β.

We could show that inhibition of TGF-β activity in experimental OA inhibited synovial fibrosis.[50] Furthermore, exposure of human fibroblasts to TGF-β or overexpression of TGF-β in murine knee joints resulted in up regulation of expression of the same genes that were found to be up regulated in synovium of knee OA patients.[57] It appears that these genes are central in synovial fibrosis and are stimulated by TGF-β. Moreover, elevated expression of procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, an enzyme increasing the number of collagen hydroxypyridinone cross links, was both detected in experimental murine OA as well as mouse knee joints with adenoviral overexpression of TGF-β.[58]

We have shown that elevated TGF-β concentrations preferentially activate the Smad1/5/8 pathway in fibroblasts.[12] In our *in vitro* experiments stimulation of fibrotic genes in fibroblast was mainly mediated by ALK5/Smad2/3 signaling. The role of ALK1/Smad1/5/8 signaling in fibrosis is not clear yet. It has been reported that ALK1 activity de-

https://doi.org/10.11005/jbm.2018.25.2.65

creases fibrosis in a number of experimental set ups.[59,60] However, on the other hand BMP9, signaling via ALK1 mainly, has been reported to be a profibrotic factor.[61] The role of ALK1, being either pro or antifibrotic, is still an enigma but can be dependent on additional factors that modulate the function of the Smad proteins.[62]

7. TGF-β and inflammation in OA

OA is long considered as a non-inflammatory wear and tear disease mainly involving cartilage degradation but is now regarded as a whole joint disease with inflammation as in integral component in many patients. TGF-β can be a stimulator of synovial inflammation and hyperplasia. It has been shown that intra-articular injection of rat knee joints with TGF-β resulted in swelling and erythema within one day.[63] The TGF-β-induced infiltrate consisted after 24 hr mostly of mononuclear phagocytes with some lymphocytes and neutrophils, while after 4 hr infiltration of mainly polymorphonuclear leukocytes was observed.[64] Antagonism of TGF-β with a neutralizing antibody in a model of erosive polyarthritis blocked cell infiltration and tissue pathology.[65] These data indicate that elated levels of TGF-β in OA joints lead to TGF-β-related inflammation that can contribute to joint damage in OA.

On the other hand, although TGF-β can attract inflammatory cells to the joint TGF-β also has a strong anti-inflammatory action. As mentioned above, mice lacking TGF-β1 develop massive inflammation resembling autoimmune disorders.[4,5,66] In lamina propria cells of the gut TGF-β suppressed nuclear factor (NF)-α-induced NF-κB p65 accumulation in the nucleus, NF-κB binding DNA activity, and NF-κB-dependent gene activation.[67] Moreover, in a mouse model of zymosan-induced arthritis injection of TGF-β had no effect on inflammation but stimulated proteoglycan content and protected against proteoglycan loss.[68] These varying result show that TGF-β attracts inflammatory cells to the joint and stimulate synovial hyperplasia and fibrosis but that it is not clear what is the end effect of TGF-β on the progression of tissue destruction. This might be related to the (Osteo) arthritis model and the timing of the TGF-β or anti- TGF-β treatment.

8. Concluding remarks

The role of TGF-β in the normal or the OA joint is strikingly different. In a normal joint TGF-β plays an important, protective, role in maintaining the differentiated chondrocyte phenotype while in an OA joint TGF-β will have a deleterious action due to its continuous presence at high levels. This changing action of TGF-β makes therapy targeting TGF-β a challenge. In general OA is a focal process, being confined to one or a defined number of joints and even in a specific joint the OA process can be at a different stage at different locations within this joint. Systemic inhibition of TGF-β has the danger of negatively affecting normal joints while the addition of extra active TGF-β can result in the pathogenic effects of TGF-β as described above. Only local inhibition in joints with severe OA appears to be an option although one has to keep in mind that TGF-β also has a role in cartilage repair as a stimulus of chondrogenesis of precursor cells. With this in mind, human trials with TGF-β or anti-TGF-β have to very well controlled, also measuring the effect of the treatment on active TGF-β levels, since expected findings can without doubt happen to the duality and context dependent action of this important cytokine.

REFERENCES

1. Wallace IJ, Worthington S, Felson DT, et al. Knee osteoarthritis has doubled in prevalence since the mid-20th century. Proc Natl Acad Sci U S A 2017;114:9332-6.
2. Newfeld SJ, Wisotzkey RG, Kumar S. Molecular evolution of a developmental pathway: phylogenetic analyses of transforming growth factor-beta family ligands, receptors and Smad signal transducers. Genetics 1999;152:783-95.
3. Dickson MC, Martin JS, Cousins FM, et al. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. Development 1995;121:1845-54.
4. Kulkarni AB, Huh CG, Becker D, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci U S A 1993;90:770-4.
5. Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 1992;359:693-9.
6. Sanford LP, Ormsby I, Gittenberger-de Groot AC, et al. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. Development 1997;124:2659-70.

7. Proetzel G, Pawlowski SA, Wiles MV, et al. Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet 1995;11:409-14.
8. Yingling JM, Wang XF, Bassing CH. Signaling by the transforming growth factor-beta receptors. Biochim Biophys Acta 1995;1242:115-36.
9. Itoh S, Itoh F, Goumans MJ, et al. Signaling of transforming growth factor-beta family members through Smad proteins. Eur J Biochem 2000;267:6954-67.
10. Finnson KW, Parker WL, ten Dijke P, et al. ALK1 opposes ALK5/Smad3 signaling and expression of extracellular matrix components in human chondrocytes. J Bone Miner Res 2008;23:896-906.
11. Blaney Davidson EN, Remst DF, Vitters EL, et al. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. J Immunol 2009; 182:7937-45.
12. Remst DF, Blaney Davidson EN, Vitters EL, et al. TGF-ss induces Lysyl hydroxylase 2b in human synovial osteoarthritic fibroblasts through ALK5 signaling. Cell Tissue Res 2014;355:163-71.
13. Cao Y, Szabolcs A, Dutta SK, et al. Neuropilin-1 mediates divergent R-Smad signaling and the myofibroblast phenotype. J Biol Chem 2010;285:31840-8.
14. Rudini N, Felici A, Giampietro C, et al. VE-cadherin is a critical endothelial regulator of TGF-beta signalling. EMBO J 2008;27:993-1004.
15. Goumans MJ, Valdimarsdottir G, Itoh S, et al. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. EMBO J 2002;21:1743-53.
16. Morales TI, Joyce ME, Sobel ME, et al. Transforming growth factor-beta in calf articular cartilage organ cultures: synthesis and distribution. Arch Biochem Biophys 1991;288: 397-405.
17. Albro MB, Nims RJ, Cigan AD, et al. Dynamic mechanical compression of devitalized articular cartilage does not activate latent TGF-beta. J Biomech 2013;46:1433-9.
18. Fava R, Olsen N, Keski-Oja J, et al. Active and latent forms of transforming growth factor beta activity in synovial effusions. J Exp Med 1989;169:291-6.
19. Fang PK, Ma XC, Ma DL, et al. Determination of interleukin-1 receptor antagonist, interleukin-10, and transforming growth factor-beta1 in synovial fluid aspirates of patients with temporomandibular disorders. J Oral Maxillofac Surg 1999;57:922-8; discussion 8-9.
20. Zielinski S, Bartels K, Cebulski K, et al. Evidence of proteolytic activation of transforming growth factor beta in synovial fluid. Adv Exp Med Biol 2000;477:477-81.
21. Hinz B. The extracellular matrix and transforming growth factor-beta1: tale of a strained relationship. Matrix Biol 2015;47:54-65.
22. Hinz B. Tissue stiffness, latent TGF-beta1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. Curr Rheumatol Rep 2009; 11:120-6.
23. Vanwanseele B, Eckstein F, Knecht H, et al. Knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. Arthritis Rheum 2002;46:2073-8.
24. Wang Q, Tan QY, Xu W, et al. Cartilage-specific deletion of Alk5 gene results in a progressive osteoarthritis-like phenotype in mice. Osteoarthritis Cartilage 2017;25:1868-79.
25. Wu Q, Kim KO, Sampson ER, et al. Induction of an osteoarthritis-like phenotype and degradation of phosphorylated Smad3 by Smurf2 in transgenic mice. Arthritis Rheum 2008; 58:3132-44.
26. Yang X, Chen L, Xu X, et al. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol 2001;153:35-46.
27. Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. J Cell Biol 1997;139:541-52.
28. van de Laar IM, Oldenburg RA, Pals G, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet 2011; 43:121-6.
29. Madej W, van Caam A, Blaney Davidson E, et al. Unloading results in rapid loss of TGFbeta signaling in articular cartilage: role of loading-induced TGFbeta signaling in maintenance of articular chondrocyte phenotype? Osteoarthritis Cartilage 2016;24:1807-15.
30. Albro MB, Nims RJ, Cigan AD, et al. Accumulation of exogenous activated TGF-beta in the superficial zone of articular cartilage. Biophys J 2013;104:1794-804.
31. Finnson KW, Parker WL, Chi Y, et al. Endoglin differentially regulates TGF-beta-induced Smad2/3 and Smad1/5 signalling and its expression correlates with extracellular matrix production and cellular differentiation state in hu-

 https://doi.org/10.11005/jbm.2018.25.2.65

man chondrocytes. Osteoarthritis Cartilage 2010;18:1518-27.
32. Hellingman CA, Davidson EN, Koevoet W, et al. Smad signaling determines chondrogenic differentiation of bone-marrow-derived mesenchymal stem cells: inhibition of Smad1/5/8P prevents terminal differentiation and calcification. Tissue Eng Part A 2011;17:1157-67.
33. Retting KN, Song B, Yoon BS, et al. BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. Development 2009;136:1093-104.
34. Valcourt U, Gouttenoire J, Moustakas A, et al. Functions of transforming growth factor-beta family type I receptors and Smad proteins in the hypertrophic maturation and osteoblastic differentiation of chondrocytes. J Biol Chem 2002;277:33545-58.
35. Zhao W, Wang T, Luo Q, et al. Cartilage degeneration and excessive subchondral bone formation in spontaneous osteoarthritis involves altered TGF-beta signaling. J Orthop Res 2016;34:763-70.
36. Day JS, Van Der Linden JC, Bank RA, et al. Adaptation of subchondral bone in osteoarthritis. Biorheology 2004;41:359-68.
37. Coughlin TR, Kennedy OD. The role of subchondral bone damage in post-traumatic osteoarthritis. Ann N Y Acad Sci 2016;1383:58-66.
38. Funck-Brentano T, Cohen-Solal M. Subchondral bone and osteoarthritis. Curr Opin Rheumatol 2015;27:420-6.
39. Zgoda M, Paczek L, Bartlomiejczyk I, et al. Transforming growth factor-beta1, interleukin-1beta and collagenase activity in subchondral bone of the femur and the severity of osteoarthritis of the hip. Clin Exp Rheumatol 2005;23:912.
40. Sakao K, Takahashi KA, Arai Y, et al. Asporin and transforming growth factor-beta gene expression in osteoblasts from subchondral bone and osteophytes in osteoarthritis. J Orthop Sci 2009;14:738-47.
41. Hayami T, Pickarski M, Wesolowski GA, et al. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum 2004;50:1193-206.
42. Zhen G, Wen C, Jia X, et al. Inhibition of TGF-beta signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med 2013;19:704-12.
43. Cui Z, Crane J, Xie H, et al. Halofuginone attenuates osteoarthritis by inhibition of TGF-beta activity and H-type vessel formation in subchondral bone. Ann Rheum Dis 2016;75:1714-21.
44. van der Kraan PM, van den Berg WB. Osteophytes: relevance and biology. Osteoarthritis Cartilage 2007;15:237-44.
45. van Beuningen HM, van der Kraan PM, Arntz OJ, et al. Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. Lab Invest 1994;71:279-90.
46. Bakker AC, van de Loo FA, van Beuningen HM, et al. Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. Osteoarthritis Cartilage 2001;9:128-36.
47. van Beuningen HM, Glansbeek HL, van der Kraan PM, et al. Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation. Osteoarthritis Cartilage 1998;6:306-17.
48. Blaney Davidson EN, Vitters EL, van Beuningen HM, et al. Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. Arthritis Rheum 2007;56:4065-73.
49. Scharstuhl A, Glansbeek HL, van Beuningen HM, et al. Inhibition of endogenous TGF-beta during experimental osteoarthritis prevents osteophyte formation and impairs cartilage repair. J Immunol 2002;169:507-14.
50. Scharstuhl A, Vitters EL, van der Kraan PM, et al. Reduction of osteophyte formation and synovial thickening by adenoviral overexpression of transforming growth factor beta/bone morphogenetic protein inhibitors during experimental osteoarthritis. Arthritis Rheum 2003;48:3442-51.
51. Blaney Davidson EN, Vitters EL, Bennink MB, et al. Inducible chondrocyte-specific overexpression of BMP2 in young mice results in severe aggravation of osteophyte formation in experimental OA without altering cartilage damage. Ann Rheum Dis 2015;74:1257-64.
52. Iwasaki M, Nakata K, Nakahara H, et al. Transforming growth factor-beta 1 stimulates chondrogenesis and inhibits osteogenesis in high density culture of periosteum-derived cells. Endocrinology 1993;132:1603-8.
53. Tuli R, Tuli S, Nandi S, et al. Transforming growth factor-

beta-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. J Biol Chem 2003;278:41227-36.
54. Eymard F, Pigenet A, Citadelle D, et al. Knee and hip intra-articular adipose tissues (IAATs) compared with autologous subcutaneous adipose tissue: a specific phenotype for a central player in osteoarthritis. Ann Rheum Dis 2017;76:1142-8.
55. Mitrovic DR, Riera H. Synovial, articular cartilage and bone changes in rapidly destructive arthropathy (osteoarthritis) of the hip. Rheumatol Int 1992;12:17-22.
56. Blaney Davidson EN, Vitters EL, Mooren FM, et al. Connective tissue growth factor/CCN2 overexpression in mouse synovial lining results in transient fibrosis and cartilage damage. Arthritis Rheum 2006;54:1653-61.
57. Remst DF, Blom AB, Vitters EL, et al. Gene expression analysis of murine and human osteoarthritis synovium reveals elevation of transforming growth factor beta-responsive genes in osteoarthritis-related fibrosis. Arthritis Rheumatol 2014;66:647-56.
58. Remst DF, Blaney Davidson EN, Vitters EL, et al. Osteoarthritis-related fibrosis is associated with both elevated pyridinoline cross-link formation and lysyl hydroxylase 2b expression. Osteoarthritis Cartilage 2013;21:157-64.
59. Morine KJ, Qiao X, Paruchuri V, et al. Reduced activin receptor-like kinase 1 activity promotes cardiac fibrosis in heart failure. Cardiovasc Pathol 2017;31:26-33.
60. Muñoz-Félix JM, Perretta-Tejedor N, Eleno N, et al. ALK1 heterozygosity increases extracellular matrix protein expression, proliferation and migration in fibroblasts. Biochim Biophys Acta 2014;1843:1111-22.
61. Muñoz-Félix JM, Cuesta C, Perretta-Tejedor N, et al. Identification of bone morphogenetic protein 9 (BMP9) as a novel profibrotic factor in vitro. Cell Signal 2016;28:1252-61.
62. Burch ML, Zheng W, Little PJ. Smad linker region phosphorylation in the regulation of extracellular matrix synthesis. Cell Mol Life Sci 2011;68:97-107.
63. Allen JB, Manthey CL, Hand AR, et al. Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta. J Exp Med 1990;171:231-47.
64. Fava RA, Olsen NJ, Postlethwaite AE, et al. Transforming growth factor beta 1 (TGF-beta 1) induced neutrophil recruitment to synovial tissues: implications for TGF-beta-driven synovial inflammation and hyperplasia. J Exp Med 1991;173:1121-32.
65. Wahl SM, Allen JB, Costa GL, et al. Reversal of acute and chronic synovial inflammation by anti-transforming growth factor beta. J Exp Med 1993;177:225-30.
66. Diebold RJ, Eis MJ, Yin M, et al. Early-onset multifocal inflammation in the transforming growth factor beta 1-null mouse is lymphocyte mediated. Proc Natl Acad Sci U S A 1995;92:12215-9.
67. Monteleone G, Mann J, Monteleone I, et al. A failure of transforming growth factor-beta1 negative regulation maintains sustained NF-kappaB activation in gut inflammation. J Biol Chem 2004;279:3925-32.
68. Glansbeek HL, van Beuningen HM, Vitters EL, et al. Stimulation of articular cartilage repair in established arthritis by local administration of transforming growth factor-beta into murine knee joints. Lab Invest 1998;78:133-42.

 https://doi.org/10.11005/jbm.2018.25.2.65