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Review

Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint stiffness

Dennis F. G. Remst[1], Esmeralda N. Blaney Davidson[1] and Peter M. van der Kraan[1]

Abstract

Synovial fibrosis is often found in OA, contributing heavily to joint pain and joint stiffness, the main symptoms of OA. At this moment the underlying mechanism of OA-related synovial fibrosis is not known and there is no cure available. In this review we discuss factors that have been reported to be involved in synovial fibrosis. The aim of the study was to gain insight into how these factors contribute to the fibrotic process and to determine the best targets for therapy in synovial fibrosis. In this regard, the following factors are discussed: TGF-β, connective tissue growth factor, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, tissue inhibitor of metalloproteinase 1, A disintegrin and metalloproteinase domain 12, urotensin-II, prostaglandin $F_{2\alpha}$ and hyaluronan.

Key words: synovium, fibrosis, osteoarthritis, TGF-β, connective tissue growth factor, lysyl hydroxylase 2b, tissue inhibitor of metalloproteinase 1, A disintegrin and metalloproteinase domain 12, Hyaluronan, $PGF_{2\alpha}$.

Rheumatology key messages

- Synovial fibrosis, which cannot be cured yet, contributes to joint pain and stiffness in OA.
- TGF-β signalling is on top of the fibrotic cascade in OA-related synovial fibrosis.
- Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 is an interesting target to block to interfere with synovial fibrosis in OA.

REVIEW

Introduction

Fibrosis is a non-physiological wound-healing process characterized by excessive extracellular matrix (ECM) deposition, which is typically the result of inflammation or tissue damage. The accumulation of excess fibrous connective tissue leads to loss of tissue homeostasis and organ failure. When synovial tissue is affected by fibrosis, which is often the case in OA, it becomes thicker and more rigid [1]. Synovial fibrosis contributes to joint pain and stiffness, which are the main symptoms of OA [2–4]. The underlying mechanisms that cause OA are still not totally unravelled, and (apart from joint replacement) no cure is available. This is an unmet need, because OA is the most common joint disease and one of the most important causes of disability in the elderly [5]. In the past, OA was considered a disease of the cartilage only. Nowadays, OA is recognized as a whole-joint disease, involving not only the cartilage, but also the subchondral bone, ligaments, meniscus and the synovium. Understanding how synovial fibrosis contributes to OA pathology and symptoms might provide avenues for future OA therapies. In this review we focus on processes/factors shown to play a role in OA-related synovial fibrosis. This will aid in choosing the best targets to interfere with OA-related fibrosis in future studies.

Synovial fibrosis in OA

The synovium can be distinguished into two different layers: the intima (synovial lining) and subintima (sublining) [6]. The intima forms an interface between the cavity containing SF and the subintimal layer. The subintima is composed of loose connective tissue and merges with the dense collagen-rich fibrous outer layer of the joint capsule.

The synovium produces SF, which is crucial for chondrocyte nutrition, and protects the cartilage from wear and tear by lubrication [6]. Multiple studies have shown that

[1]Radboud University Medical Center, Experimental Rheumatology, Nijmegen, The Netherlands

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Correspondence to: Peter M. van der Kraan, Radboud University Medical Center, Experimental Rheumatology, Geert Grooteplein Zuid 26-28, 6525 GA Nijmegen, The Netherlands.
E-mail: Peter.vanderKraan@radboudumc.nl

the synovia of patients suffering from early or advanced OA have some form of pathology [7–10]. Synovial pathology may impair joint functionality and contribute to disease progression by, for example, increased joint friction [7]. Oehler *et al.* [9] divided osteoarthritic synoviopathy into four different subtypes based on the nature of the synovium: hyperplastic, inflammatory, fibrotic or detritus-rich. Fibrosis was abundantly present in both the fibrotic and detritus-rich synoviopathy and only to a minor extent in the inflammatory subgroup. Instead of fibrotic and detritus-rich synoviopathy, we will use the more general term synovial fibrosis for both synoviopathies in the remainder of this article. Although dividing synoviopathy into different subtypes may help in grouping OA patients and/or disease progression, we have to keep in mind that the observation of synovial fibrosis at different time points is patient and site dependent. Moreover, in most cases inflammation and fibrosis can co-exist and are interdependent.

Kerna *et al.* [11] reported an enhanced level of inflammation, lining layer thickness, number of $CD4^+$ T cells and macrophage infiltration in patients with very early OA compared with late-stage OA. This confirms the observation by Oehler *et al.* [9] that in early OA more inflammation was present, whereas in late-stage OA more fibrosis was observed. These outcomes also support the study of Haraoui *et al.* [8], who reported that the amount of fibrosis is inversely proportional to the extent of cellular infiltrate in the OA synovium, and that fibrosis is mainly but not exclusively found in late-stage OA. These results indicate a shift from the inflammatory to the fibrotic subgroup, which may suggest that the factors inducing fibrosis are upregulated in the inflammatory phase.

Factors involved in synovial fibrosis

A vast number of factors can contribute to fibrosis, many of which are cell type or disease specific. Therefore, we performed a search for synovial fibrosis OA via PubMed (limited to 2008–2015). This yielded 45 results. Factors only found to be induced at the mRNA level were omitted from the list, and the following factors were reported to be elevated in humans with OA-related fibrosis: TGF-β, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2) [also known as lysyl hydroxylase 2b (LH2b)], tissue inhibitor of metalloproteinase 1 (TIMP-1), A disintegrin and metalloproteinase domain 12 (ADAM12), prostaglandin F2α ($PGF_{2\alpha}$), urotensin-II and mammalian target of rapamycin (mTOR) [11–16]. From this list, PLOD2, TIMP-1 and mTOR have also been shown to be elevated in experimental OA models [12, 16]. Furthermore, lysophosphatidic acid has also been found to be elevated in experimental OA [17]. Because we focused on the synovium, mTOR and lysophosphatidic acid were not described in greater detail, as these factors were only found to be elevated in chondrocytes/cartilage and not in synovial fibroblasts or the synovium. Connective tissue growth factor (CTGF) was added to the list, because this is a well-known fibrotic factor that has also been shown to induce synovial fibrosis.

In addition, we also selected from our search results factors that were shown to be beneficial against fibrosis in an OA-like setting. Hyaluronan, polysulphated glycosaminoglycan, parathyroid hormone and Stanozolol were reported to be protective against OA-related fibrosis (Table 1) [18–22]. We choose to describe hyaluronan in more detail because this factor has been found to be effective against OA-related fibrosis by multiple groups in a range of species, whereas the other factors have only been described by one group and for one species. For all the selected factors, additional and background information was acquired via PubMed.

TGF-β signalling: central in the fibrotic cascade

TGF-β is the most well known and best described fibrotic factor and a key player in many profibrotic processes, including epithelial mesenchymal transition, enhancing expression of TIMPs and elevating ECM deposition [23, 24]. To our knowledge, TGF-β has been found involved/elevated in all fibrotic tissues researched so far, for example (but not exclusively): in fibrotic lesions of liver, lung, kidney, skin and heart tissue [25, 26]. Furthermore, it has been shown in various fibrotic settings that inhibition of TGF-β signalling attenuates fibrosis, whereas overexpression of TGF-β causes fibrosis [25, 27, 28].

Ideally, to prevent fibrosis, one would like to block TGF-β, the top of the fibrotic cascade. However, TGF-β is a regulator of many crucial cellular processes. Blocking TGF-β would result in serious side effects and thus cannot be considered the ultimate cure for fibrosis. Therefore, it is important to identify targets downstream of TGF-β that drive fibrosis in order to minimize unwanted side effects. To identify these downstream targets of TGF-β for fibrosis therapy, one should first understand how TGF-β signals in fibrosis. It is now common knowledge that TGF-β, by binding the TGF-β type II receptor, can signal via two distinct type one receptors, namely ALK5 and ALK1, which in turn phosphorylate receptor Smads, Smad2/3 and Smad1/5/8, respectively [29]. The receptor Smads can form complexes with the common Smad (Smad4) and translocate to the nucleus to induce gene transcription.

The role of ALK1 in fibrosis is not completely clear, and the literature on this seems to be inconsistent. For instance, in irradiation-induced kidney fibrosis, $ALK1^{+/-}$ mice developed less inflammation and fibrosis at 20 weeks after irradiation compared with wild-type littermates [30]. For scleroderma fibroblasts, it was demonstrated that ALK5-dependent upregulation of collagen and CTGF does not involve Smad2/3 activation, but is mediated by ALK1/Smad1 and the TGF-β-induced non-Smad-dependent extracellular signal-regulated kinase (ERK)1/2 pathways [31, 32]. These observations indicate a profibrotic role for ALK1. However, $ALK1^{+/-}$ mice with ureteral unilateral obstruction–induced kidney fibrosis showed (after 15 days) significantly higher expression of type I collagen compared with wild-type mice [33]. Furthermore, cultured renal fibroblasts from $ALK1^{+/-}$ mice expressed more collagen type I and fibronectin

Table 1 Factors found to be protective against OA-related fibrosis

Factors found to be protective against OA-related fibrosis	Species	Proposed/possible mechanism to reduce fibrosis
Hyaluronan	Ovine, horse, mice	See section on hyaluronan
Polysulphated glycosaminoglycan	Horse	Decrease in inflammatory mediators
Parathyroid hormone	Rabbits	Inhibition of collagen, type 1, alpha 1
Stanozolol	Ovine	Reduced inflammatory phase

than fibroblasts derived from wild-type mice. These results indicate a more anti-fibrotic role for ALK1, which is in contrast to the studies mentioned above. Apparently, within one organ, like kidney, the use of a different model system can result in a different outcome. This suggests that the role of ALK1 is not only cell type and tissue dependent, but may also be influenced by the ailment of the tissue [34].

ALK5-mediated signalling is known to induce most of TGF-β's profibrotic effects, and inhibition of ALK5 has been shown to repress fibrosis in several fibrotic diseases [35–37]. Because ALK5 signals via both Smad2 and Smad3, which can potentially have different effects, their individual roles in fibrosis have been investigated, most frequently in epithelial cells. In these cells, Smad3 is profibrotic, whereas Smad2 protects against Smad3-mediated fibrosis [38–40]. Because the specific roles of either Smad2 or Smad3 can be tissue dependent, the individual functions of Smad2 and Smad3 in the synovium have yet to be determined.

Besides the TGF-β–Smad pathways, which are well described in general and specifically regarding fibrosis, there are also Smad-independent TGF-β signalling pathways. The Smad-independent TAK-1 pathway has been shown to have profibrotic effects in regulating the expression of ECM proteins, including collagens and fibronectin [41]. Furthermore, in a TGF-β-driven murine model of dermal fibrosis, inhibition of TGF-β-dependent ERK phosphorylation showed strong and dose-dependent antifibrotic effects on skin thickening [42]. This indicates that not only the TGF-β–Smad pathways, but also the Smad-independent TGF-β signalling pathways have profibrotic properties. Unfortunately, not much is known about these Smad-independent TGF-β signalling pathways concerning synovial fibrosis and their functions in the synovium, which puts limitations on selecting the optimal target to interfere with synovial fibrosis. These non-Smad signalling factors are central mediators in multiple pathways, which makes their mechanism of action very elaborate, and therefore they are potentially less suitable as targets to interfere with synovial fibrosis.

CTGF—TGF-β's right hand in the fibrotic cascade

CTGF is also known as CCN family protein 2 (CCN2). A primary function of CTGF is to modulate and coordinate signalling responses involving cell surface proteoglycans (key components of the ECM and growth factors) [43]. During adulthood, CTGF is expressed in endothelia and neurons in the cerebral cortex, where it promotes angiogenesis and tissue integrity, and in the female reproductive tract, where it regulates both follicle development and ovulation [44–46]. In addition, CTGF is expressed in wound healing, vascular diseases and fibrosis [47–49].

CTGF, like TGF-β, is found to be elevated in many fibrotic diseases. There is no unique receptor known for CTGF to which it binds with high affinity, and therefore CTGF is considered a matricellular protein that modulates the interaction of cells with the matrix, which modifies the cellular phenotype [50]. It is suggested by Leask and Abraham [51] that CTGF mediates its effects through integrin- and heparin sulphate proteoglycan–dependent mechanisms, and that the ability of CTGF to bind cell surface heparin sulphate proteoglycans (which are present at high levels in the joint) is essential for CTGF activity. Because, no data are available about the interaction between TGF-β and CTGF in the synovium, we will discuss what is in our opinion the best alternative data: that for cellular signalling responses in fibroblasts in other tissues.

As CTGF is a potent enhancer of fibroblast proliferation, chemotaxis and ECM deposition, CTGF is thought to mediate some of the fibrogenic effects of TGF-β after being upregulated by TGF-β [43, 52]. Furthermore, CTGF decreases Smad7, an inhibitory Smad that can inhibit TGF-β signalling on multiple levels and, via this mechanism, promote TGF-β signalling [53]. The mechanism by which CTGF regulates Smad7 is not yet fully unravelled. However, one proposed mechanism is by induction of TIEG-1, which is upregulated via the TrkA signalling receptors for CTGF [54, 55]. Depletion of CTGF in foreskin fibroblasts via adenoviral CTGF siRNA almost completely abrogated TGF-β-induced upregulation of collagen synthesis, indicating that CTGF not only enhances some of the profibrotic effects of TGF-β, but is also obligatory for certain profibrotic effects [56].

Where others have demonstrated that only the combination of TGF-β and CTGF leads to persistent fibrosis, we have previously published that overexpression of TGF-β alone causes persistent synovial fibrosis, whereas CTGF alone in the murine knee joint causes only transient synovial fibrosis [27, 28, 57, 58]. Because TGF-β is a potent inducer of CTGF, we cannot rule out the possibility that it is essential for the induction of persistent synovial fibrosis. It has been suggested by Wang *et al.* [28] that the threshold level of CTGF necessary to induce persistent fibrosis may not be always reached by injecting TGF-β alone. Therefore, a possible explanation for this discrepancy

might be that overexpressing Ad-TGF-β results in higher levels of TGF-β and subsequently higher CTGF levels compared with that achieved by protein injection of TGF-β, and therefore might reach the CTGF threshold required to induce persistent synovial fibrosis. In addition, both the synovial fibroblasts and the chondrocytes in the cartilage strongly induce CTGF expression upon TGF-β stimulation [14, 59]. Our finding that CTGF can cause transient fibrosis is in line with the observation that CTGF by itself can promote collagen synthesis. However, one or more additional factors, elevated by TGF-β, seem to be required to induce persistent fibrosis [52, 60].

To validate CTGF as a potential antifibrotic target, it is important to determine whether CTGF is necessary for the persistence of TGF-β-induced synovial fibrosis, especially since a CTGF blocking antibody (FG-3019) is available. This antibody attenuated the fibrotic response in three independent models of fibrosis: a model of multiorgan fibrosis induced by repeated i.p. injections of CTGF and TGF-β, a unilateral ureteral obstruction renal fibrosis model and an intratracheal bleomycin instillation model of pulmonary fibrosis [28].

PLOD2

PLOD2 is a collagen cross-linking enzyme, which activity induces the formation of pyrodinoline cross-links [61]. Increased expression of *Plod2* mRNA is found in a range of fibrotic fibroblasts [62]. Also the pyrodinoline cross-links, which make collagen fibrils less susceptible to enzymic degradation and more rigid, are found to be elevated in various fibrotic tissues [62]. Diminished collagen degradation resulting from increased pyridinoline cross-links per collagen triple helix, results in collagen accumulation, which is one of the hallmarks of fibrosis. One of the most potent inducers of PLOD2 is TGF-β [14, 63]. However, for skin fibroblasts it was shown that IL-4, BMP-2, activin A and TNF-α can also enhance PLOD2 expression [63]. For synovial fibroblasts, it was shown that besides TGF-β, $PGF_{2\alpha}$ also induces PLOD2 expression [13].

We observed in OA-induced fibrosis that both PLOD2 expression and the number of pyridinoline cross-links per collagen triple helix in the synovium were elevated [27]. Most importantly, we also found elevated levels of PLOD2 in human end-stage OA synovium [12]. Because the presence of fibrosis in these OA patients was unknown, the average PLOD2 level might be even higher in the subpopulation of OA patients with fibrosis. This elevation suggests that PLOD2 may be crucial in OA-related synovial fibrosis. To our knowledge no blocking or overexpression studies of PLOD2 currently exist that determine its direct function in the fibrotic process. However, based on the function of PLOD2 and the fact that it is highly induced in OA synovium, PLOD2 is an appealing target for study regarding its potential interference with synovial fibrosis.

TIMP-1

TIMP-1 is an inhibitor of the MMPs—peptidases involved in ECM degradation—and is found to be elevated in a number of fibrotic diseases, for example, pulmonary, liver and kidney fibrosis [64–66]. We found that TIMP-1 is elevated in the synovium of both human end-stage OA patients and mice with experimental OA [12]. TIMP-1 is induced by TGF-β and is typically proposed as an enhancer of fibrosis development, but does not induce fibrosis itself [65, 66]. Inhibition of TIMP-1 is expected to result in higher MMP activity and therefore more ECM breakdown, which might be beneficial in diminishing fibrosis. However, in unilateral urethral obstruction–induced fibrosis, there was no difference in the degree of interstitial fibrosis detected between wild-type and TIMP-1–deficient mice [67]. Most likely the role of TIMP-1 may vary between the various types of fibrosis, and its role in synovial fibrosis has yet to be discovered.

Urotensin II

Urotensin II is a potent vasoconstrictor that is involved in cardiac remodelling, and it may influence cardiovascular homeostasis and pathology [68, 69]. Furthermore, it may also influence the CNS and endocrine function in man [69]. In various fibrotic diseases, for example, hepatic, pulmonary and cardiac fibrosis, urotensin II levels are elevated [70–73]. Moreover, the authors of these articles suggest that urotensin II is involved in the development of fibrosis. Most fascinatingly, urotensin II levels were also reported to be significantly higher in the SF of OA patients compared with controls, and thus may be associated with synovial fibrosis in OA [15]. It is reported that urotensin II may stimulate collagen synthesis via the ERK1/2 and TGF-β/Smad2/3 signalling pathway and may in this way contribute to fibrosis [68, 74]. The exact signalling mechanism of urotensin II is, however, largely unknown. Therefore, more knowledge is needed about the interplay between urotensin II and TGF-β signalling in synovial fibroblasts and about its potential role in synovial fibrosis.

ADAM12

ADAM12 is primarily involved in cell adhesion and fusion, ECM restructuring and cell signalling. There are two different splice variants: a shorter secreted form (ADAM12-S) and a longer membrane-bound form (ADAM12-L) [75]. Elevated serum levels of ADAM12-S are associated with elevated serum inflammatory markers, severity of skin fibrosis and increased activity of interstitial lung disease in dcSSc, suggesting a profibrotic role for ADAM12 [76]. Furthermore ADAM12-L was found to be elevated in the cartilage of OA patients [77]. Most interestingly, both ADAM12-S and ADAM12-L were upregulated in the synovial tissue of patients with OA and positively correlated with the grade of synovial fibrosis, suggesting a role for ADAM12 in OA-related synovial fibrosis [11, 77].

ADAM12 is potently induced by TGF-β at both mRNA and protein level in various cell types, including fibroblasts, enhancing epithelial to mesenchymal transition, α-smooth muscle actin (α-SMA) expression and ECM production [78–80]. The proposed mechanism by which ADAM12 induces its profibrotic effects is by positively

regulating TGF-β signalling, due to stabilization of the TβRII protein [81]. This stabilization might be accomplished by suppressing the association of TβRII with Smad7, thus preventing degradation of the receptor complex by Smad7 [81, 82]. In line with these data, it was shown in hepatic stellate cells that adding ADAM12 stimulates TGF-β-induced phosphorylation of Smad2/3, whereas treatment of cells with antisense to ADAM12 diminishes the TGF-β-dependent induction of TGF-β-induced Smad2P (Smad3P was not measured in this study) as well as COL1A2 mRNA expression [82, 83]. ADAM12 could therefore be an important modulator of TGF-β-induced fibrosis.

$PGF_{2\alpha}$

$PGF_{2\alpha}$ normally regulates a number of important physiological functions, like uterine contraction and bronchoconstriction. However, elevated plasma concentrations of $PGF_{2\alpha}$ metabolites found in idiopathic pulmonary fibrosis are significantly associated with both disease severity and prognosis [84]. Oga *et al.* [85] have shown that both $PGF_{2\alpha}$ and TGF-β increased the promoter activity of COL1A2, and simultaneous addition of both factors synergistically increased the COL1A2 promoter activity. Furthermore, $PGF_{2\alpha}$ deficiency and inhibition of TGF-β signalling additively decrease fibrosis in mice with idiopathic pulmonary fibrosis, suggesting that TGF-β and $PGF_{2\alpha}$ recruit different signalling molecules to induce collagen production [85]. These results indicate that $PGF_{2\alpha}$ has profibrotic effects that work independently of TGF-β.

The $PGF_{2\alpha}$ isoforms 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were found to be significantly increased in the SF of patients with OA [86]. Also, relatively high levels of $PGF_{2\alpha}$ were measured in infrapatellar fat pad (from OA patients)–conditioned medium (FCM) [13]. Collagen production by fibroblast-like synoviocytes was positively associated with $PGF_{2\alpha}$ levels in this FCM. In addition, gene expression of the collagen cross-linking gene, *Plod2* was increased in fibroblast-like synoviocytes in the presence of this FCM. Inhibition of $PGF_{2\alpha}$ levels reduced the extent of FCM-induced collagen production and *Plod2* expression, whereas inhibition of the TGF-β–ALK5 pathway with SB505124 did not alter the FCM-induced effects on fibroblast-like synoviocytes. These results indicate that elevated levels of $PGF_{2\alpha}$ and its isoforms are present in an OA joint, and that $PGF_{2\alpha}$ has profibrotic effects on the synovium that might differ from those induced by TGF-β.

Hyaluronan

Hyaluronan is a glycosaminoglycan that binds to the CD44 receptor. Injection of hyaluronan 24 h after TGF-β injection in the TGF-β prior to treadmill running model of OA inhibited the cascade of OA-like joint changes, including gait changes and synovial fibrosis. Furthermore, hyaluronan injection post-surgery in the meniscectomy-induced OA model in sheep reduced synovial fibrosis [22]. These results show that hyaluronan protects against OA-related fibrosis in both IA injection of TGF-β prior to the treadmill running model of OA and the meniscectomy-induced OA model in sheep [21, 22]. This is in agreement with the observation that exogenously provided hyaluronan antagonized TGF-β1-dependent myofibroblast differentiation [87]. However, the exact mechanism by which hyaluronan interferes with synovial fibrosis is unknown. One mechanism suggested by Plaas *et al.* [88] is that hyaluronan may act as an antifibrotic by blocking ADAMTS5-mediated activation of profibrotic pathways in peri-articular cells. This, because hyaluronan can form a complex with Adamts5, and ablation of Adamts5 has been shown to prevent both cartilage erosion and fibrotic remodelling in challenged joints [89]. Another possible explanation is that interaction of hyaluronan with its receptor results in an increase in the association of the TGF-β receptor with Smad7, leading to TGF-β receptor degradation [81]. This degradation leads to a decrease in TGF-β signalling and therefore in less fibrosis. That hyaluronan may be beneficial in the reduction of fibrosis by attenuating TGF-β signalling again suggests a major role for TGF-β signalling in fibrosis.

Targets to block synovial fibrosis in OA

The main cause of synovial fibrosis seems to be TGF-β–ALK5 signalling. Unfortunately, blocking ALK5 may not be without any consequences for the cartilage, because blocking ALK5 has been shown to promote MMP13 expression and diminish type II collagen expression in chondrocytes [90, 91]. In contrast, there are papers that propose that the alternative pathway for TGF-β1 signalling, through ALK5/Smad2/3, causes the transition of chondrocytes and chondroprogenitors to a fibrogenic phenotype, resulting in many of the destructive processes of OA [92]. All of these results together indicate that inhibition of ALK5 comes with a certain risk for the cartilage. Therefore it is better not inhibited in an OA joint unless it is specifically blocked in the synovium to prevent fibrosis, which is unfortunately not yet possible.

Whether inhibition of ALK1 in an OA joint has pro- or antifibrotic effects remains to be elucidated. This may be worth investigating, because besides the potential antifibrotic effects, inhibition of ALK1 is expected to reduce MMP13 expression in chondrocytes and therefore MMP-mediated cartilage damage—a potential win–win situation [90, 91]. However, to minimize potential side effects, inhibition of a gene with a single function (or limited functions) is preferrred over blocking genes with multiple functions or those that are at the top of an extensive signalling pathway, such as TGF-β or $PGF_{2\alpha}$. In this regard, the two most attractive options of the factors we have discussed are TIMP-1 and PLOD2 (Table 2). A major drawback of targeting TIMP-1 in an OA joint is that the elevated MMP activity will contribute to cartilage damage [100]. Therefore, inhibition of TIMP-1 in an OA joint is not the preferred option for interfering with OA-related synovial fibrosis. PLOD2, on the other hand, is a potential target for blockade in synovial fibrosis. Notably, cartilage containing high levels of

Table 2 Pros and cons of inhibiting CTGF, PLOD2 or TIMP-1 for the synovium and cartilage

		Expected effect on the synovium		Expected effect on the cartilage
CTGF inhibition	Pro	Less collagen synthesis might attenuate the inflammatory cascade [52, 60, 93]	Pro	May prevent CTGF-mediated joint/ cartilage destruction in OA [57]
	Con	Unknown	Con	CTGF/CCN2 may play a role as an anti-ageing factor by stabilizing articular cartilage [57, 93, 94]
PLOD2 inhibition	Pro	Less pyridinoline cross-links in the collagens triple-helixes. Prevents that he collagen becomes harder to degrade and therefore prevents long lasting collagen accumulation [27, 62, 63, 95].	Pro	Cartilage areas containing low pyridinoline levels are less prone to degeneration compared with cartilage containing high levels of pyridinoline collagen cross-links, which seems to fail mechanically under long-term loading [96]
	Con	Unknown	Con	Unknown
TIMP-1 inhibition	Pro	More collagen degradation (as a result of more MMP activity) may reverse the fibrosis [97]	Pro	Unknown
	Con	Unknown	Con	More collagen degradation (due to more MMP activity), causing more cartilage damage [98, 99]

pyridinoline collagen cross-links, which are increased due to PLOD2 activity, seems to fail mechanically under long-term loading, whereas areas containing low pyridinoline levels are less prone to degeneration [96]. This suggests that inhibition of PLOD2, besides the potential antifibrotic effects, may also favour cartilage repair in an OA joint. We look forward to an experiment where PLOD2 is blocked in an OA model accompanied by fibrosis in order to determine whether this approach indeed prevents synovial fibrosis.

Discussion

Because it is estimated that over half of all OA patients suffer from synovial fibrosis, it is important that this pathological process receives more attention, especially as fibrosis is one of the main causes of joint stiffness [2–4, 101]. At present, there are no options for interfering with synovial fibrosis; however, preventing or reversing fibrosis in OA might result in major symptom relief. The goal of this review was to provide an overview of the known factors that play a role in initiating and sustaining synovial fibrosis so as to facilitate the selection of targets for antifibrotic therapies.

Several factors can contribute to excessive deposition of the ECM and the resultant synovial fibrosis, either by increasing ECM synthesis or by decreasing its degradation. The majority of these profibrotic factors are either downstream of TGF-β or modulate TGF-β signalling. There are other pathways that may contribute to synovial fibrosis independently of TGF-β, for instance $PGF_{2\alpha}$. It is hard to predict the relative contribution of these factors to the fibrotic process. Blockade studies are required in order to elucidate whether inhibition of one of these factors will break the fibrotic cascade in synovial fibrosis.

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