Effects of cytokine signaling inhibition on inflammation-driven tissue remodeling

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1. Background

Fibrosis is a common condition that can affect all body tissues, driven by unresolved tissue inflammation and resulting in tissue dysfunction and organ failure that could ultimately lead to death. A myriad of factors are thought to contribute to fibrosis and, although it is relatively common, treatments focusing on reversing fibrosis are few and far between. The process of fibrosis involves a variety of cell types, including epithelial, endothelial, and mesenchymal cells, as well as immune cells, which have been shown to produce pro-fibrotic cytokines. Advances in our understanding of the molecular mechanisms of inflammation-driven tissue fibrosis and scar formation have led to the development of targeted therapeutics aiming to prevent, delay, or even reverse tissue fibrosis. In this review, we describe promising targets and agents in development, with a specific focus on cytokines that have been well-described to play a role in fibrosis: IL-1, TNF-α, IL-6, and TGF-β. An array of small molecule inhibitors, natural compounds, and biologics have been assessed in vivo, in vivo, and in the clinic, demonstrating the capacity to either directly interfere with pro-fibrotic pathways or to block intracellular enzymes that control fibrosis-related signaling pathways. Targeting pro-fibrotic cytokines, potentially via a multi-pronged approach, holds promise for the treatment of inflammation-driven fibrotic diseases in numerous organs. Despite the complexity of the interplay of cytokines in fibrotic tissues, the breadth of the currently ongoing research targeting cytokines suggests that these may hold the key to mitigating tissue fibrosis and reducing organ damage in the future.

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establishing an effective treatment to prevent or resolve is essential for improving organ function and overall quality of life in patients. For this reason, multiple drugs and inhibitors are currently in development for treating fibrosis in specific organs. However, due to the complexity of the pathways involved and the various functions of these organ, many treatments are not effective in all cases of fibrosis. In an effort to address the inflammatory etiology of organ fibrosis, a number of investigations have been performed to assess the utility of cytokine inhibition in mitigating organ fibrosis. These efforts have mainly focused on four cytokine families: IL-1, TNF-α, IL-6 and TGF-β. This review will describe the roles of these cytokines in the development of fibrosis and highlight utility of cytokine inhibition in reversing tissue scarring (Fig. 1).

2. The IL-1 family

Interleukins are signaling molecules produced by leukocytes and are crucial mediators of the immune response. Many members of the IL-1 family are regarded as pro-inflammatory interleukins and therefore increase the immune response to stimuli. This increase could drive inflammation and exacerbate fibrosis. Reversing this effect using IL-1 inhibitors may reduce fibrosis in the affected organ (Fig. 2).

IL-1β is the most abundant cytokine in the IL-1 family and circulates as the precursor pro-IL-1β. The pro-IL-1β protein is matured by the NLRP3 inflammasome and the activation of caspase-1 (He et al., 2016). Cáceres et al. (2019) inhibited the NLRP3 inflammasome using the drug serelaxin, which is a recombinant form of the protein relaxin. They demonstrated that serelaxin treatment of cardiac myofibroblasts inhibited TLR4, which resulted in the NLRP3 inflammasome being unable to form and therefore IL-1β remaining in its inactive form. They also suggested that this prevented myofibroblast differentiation and reduced collagen deposition. This study implied that in vivo treatment with serelaxin may reduce remodeling and scar tissue formation in cardiac fibrosis (Cáceres et al., 2019). Serelaxin has also been used to treat fibrosis in an OVA-driven model of allergic airway disease. It was able to reduce fibrosis and facilitate bronchodilation, likely through its ability to encourage collagen turnover through an increase in MMPs, which along with the degradation of ECM proteins, is able to reduce the effect of other pro-fibrotic cytokines (Lam et al., 2016, 2018; Royce et al., 2009).

As well as serelaxin, studies have suggested that the drug Empagliflozin also targets the formation of the NLRP3 inflammasome. Sukhanov et al. identified the role of another cytokine (IL-17) on inflammasome formation and subsequent IL-1β activation (Sukhanov et al., 2021). They showed that, through the inhibition of IL-17, various downstream effects can be attenuated, such as the migration and proliferation of aortic smooth muscle cells in vascular proliferative disease. The association between IL-17 and IL-1β activation was confirmed through an increase of IL-1β mRNA and secreted protein, which subsequently induced cell migration and proliferation. They also highlighted that the relationship between IL-17 and the NLRP3 inflammasome may be dependent on NF-κB and AP-1, which are mediated by TRAF3IP2. Empagliflozin treatment was shown to prevent the increase of IL-1β and NLRP3 expression and reduce the proliferation and migration that was induced by IL-17 (Sukhanov et al., 2021). Targeting the proliferation and migration that is seen in vascular proliferative disease as well as many

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other fibrotic conditions by drugs such as empagliflozin could reduce the damaging effect mesenchymal cells have on inflamed tissue.

A natural inhibitor of IL-1β also exists, known as IL-1Ra, which may be utilized in its recombinant form (anakinra) to reverse fibrosis. It acts as a competitive inhibitor and prevents the activation of the IL-1R1 receptor, therefore preventing the activation of NF-κB via IL-1β. A recent in vivo study was performed on mice with alcohol-induced liver disease showing that treatment with recombinant IL-1Ra reduced organ fibrosis, assessed by evaluating the expression of PIIINP, a form of procollagen that is often used as a marker for fibrosis (Lotfy et al., 2019). These results corroborated an earlier study showing that anakinra reduced levels of IL-1β in mice with alcoholic liver disease (Petrasek et al., 2012), indicating that this inhibitor may be a potential treatment for alcohol-induced liver fibrosis.

Another study testing an inhibitor targeting the IL-1β pathway in liver fibrosis was performed on mice with non-alcoholic steatohepatitis. Barreyro et al. (2015) used the pan-caspase inhibitor emricasan to target caspase-1, one of the essential proteins needed for IL-1 maturation. By assessing markers of liver damage, i.e. aspartate aminotransferase and alanine aminotransferase, these investigators suggested that emricasan can reverse liver fibrosis induced by a high-fat diet. Inflammatory markers were also tested for, including IL-1β, with emricasan-treated mice expressing significantly less IL-1β when measured by qPCR (Barreyro et al., 2015). This suggests that it is possible to inhibit IL-1β by preventing its maturation and forcing it to remain in the inactive pro-IL-1β form.

The arthritis drug auranofin has also been investigated in the treatment of non-alcoholic fatty liver disease (Hwangbo et al., 2020). Through staining of liver tissue in a mouse model of fatty liver disease, it was shown that auranofin treatment reduced the number of circulating macrophages, which would decrease the amount of inflammatory cytokines secreted. It was confirmed via Western blot that auranofin reduced the secretion of caspase-1 and subsequently IL-1β compared to the control. This was also shown to be due to the reduction of NLRP3
inflammation following treatment. Auranoﬁn likely interrupts the for-
mation of the NLRP3 inﬂammosome in ﬁbrotic liver tissue resulting in
the activation of IL-1β by caspase-1 (Hwangbo et al., 2020). The up-
stream target of auranoﬁn encourages a widespread effect and increased
the effectiveness of the drug.

An existing drug, artesunate, normally used to treat malaria has been
shown to combat ﬁbrotic diseases, speciﬁcally hepatic ischemia
(El-Sayed Ghoneim et al., 2020). It has been shown that treatment of
artesunate in rats with hepatic ischemia reduced the expression of the
NLRP3 protein complex as well as caspase-1, the protein responsible for
the activation of IL-1β. This was conﬁrmed via Western blot as well as
the subsequent reduction of circulating IL-1β. This downstream inhibition
was likely to be determined by the disruption of TLR4, TRAF6 and NF-κB
signaling shown via ELISA assay. It was also shown that artesunate has an
overall protective effect against hepatic ischemia as liver injury scores
determined through histopathological staining were signiﬁcantly low-
ered (El-Sayed Ghoneim et al., 2020). The repurposing of existing drugs
to treat ﬁbrosis is a lucrative pathway in drug development as thorough
safety analyses have already been performed.

Studies have also focused on IL-1β in renal ﬁbrosis. Masola et al.
(2019) performed an in vitro study using human renal proximal tubular
epithelial cells (HK-2) and hepatic stellate cells and (LX-2, i.e. pericytes)
to test the impact of IL-1β inhibition on ﬁbrosis. This study focused on
epithelial-to-mesenchymal transition (EMT) as a driver of ﬁbrosis and a
source of myoﬁbroblasts, which is induced by the release of inﬂamma-
tory cytokines and growth factors and leads to the overproduction of
collagen and tissue remodeling (Fragiadaki and Mason, 2011). It was
observed that treatment with IL-1β induced EMT, demonstrated by
increased expression of the ﬁbrotic markers ﬁbronectin, vimentin and
α-smooth muscle actin (α-SMA). Crucially, treatment with the IL-1β in-
hibitor canakinumab reversed the expression of these markers as well as
that of a critical mediator of EMT, MPP-2. Moreover, canakinumab also
attenuated the induction of a myoﬁbroblast phenotype caused by TGF-β1
treatment, suggesting that the inhibition is not speciﬁc to IL-1β, indi-
cating that this therapeutic modality may also be promising for resolving
kidney ﬁbrosis (Masola et al., 2019).

Renal ischemia also results in inﬂammation, oxidative stress and the
induction of ﬁbrosis. Taraxasterol, the active compound in the traditional
herbal medicine Taraxacum ofﬁcinalis, has been investigated for its anti-
inﬂammatory effects (Li et al., 2020). In mice with acute kidney injury,
taraxasterol treatment decreased the pathological score of renal tubular
injury, indicating a reduction in cell necrosis, tubular dilation and the
inﬁltration of inﬂammatory cells. This suggests that taraxasterol must
interrupt upstream inducers of ﬁbrosis. In this model of kidney injury,
macrophage numbers signiﬁcantly increased along with interstitial
ﬁbrosis; these were both attenuated with taraxasterol treatment, along
with a reduction in serum IL-1β. This was likely achieved by a reduction in
ERK and JNK phosphorylation, thus blocking the downstream effects of
IL-1β. It was also suggested that reactive oxygen species are produced
following IL-1β signaling, which was abrogated by taraxasterol via the
inhibition of MAPK signaling (Li et al., 2020). The combined approach
with taraxasterol mitigating both inﬂammation and oxidative stress
seems to be a promising mode of action in reducing tissue remodeling
in ﬁbrosis.

Another natural anti-ﬁbrotic drug being explored is andrographolide,
a compound isolated from Andrographis paniculata; this natural resource
has been traditionally used to treat inﬂammation, although the mecha-
nism of action is not clear. Gupta et al. investigated the effects of this
active compound in a mouse model of arthritis manifested as an increase
in the inﬁltration of immune cells in the hind limbs (Gupta et al., 2020).
Histopathological analysis suggested that treatment with andrographo-
lide reduced the number of immune cells present and encouraged the
regrowth of the synovial lining. Mechanistically, it was found that
andrographolide attenuated NF-κB translocation into the nucleus,
thereby inducing immune suppression. Along with this, the phosphory-
lation of p38 and the concentration of serum IL-1β were signiﬁcantly
reduced following andrographolide treatment. This inhibition was
shown to decrease paw edema and improve the presentation of arthritic
ﬁbrosis (Gupta et al., 2020). By targeting NF-κB, which is involved in
many pro-inﬂammatory and pro-ﬁbrotic signaling cascades, androgra-
pholide may have a beneﬁcial impact in a number of inﬂammatory and
ﬁbrotic diseases.

IL-1β has also been identiﬁed as one of the most signiﬁcant triggers in
ﬁbrosis following myocardial inﬁarction. It has been highlighted that IL-
1β may contribute to ﬁbroblast differentiation, which would contribute
to disordered tissue remodeling. Treating mice with the small molecule
MCRB-613, a steroid receptor coactivator stimulator, was shown to
decrease IL-1β mRNA expression in cardiac ﬁbroblasts following
myocardial infarction via single cell analysis (Mullany et al., 2020). This
was also shown in an inﬂamed microenvironment induced by M1 mac-
rophages. Mullany et al. suggested that MCRB-613 works via the upregu-
lation of SRC-3, which is an inhibitor of IL-1β activity in macrophages.
This, in turn, would reduce the concentration of IL-1β in the microenvi-
ronment, thus reducing the differentiation of cardiac ﬁbroblasts. This
was mechanistically conﬁrmed with in vitro experiments. Treatment
with MCRB-613 was shown to greatly reduce ﬁbrotic tissue in murine
hearts following myocardial infarction, illustrating the overall effect of
IL-1β inhibition and the reduction of inﬂammation in the microenvi-
ronment (Mullany et al., 2020). This study highlights the contribution of
ﬁbroblasts in cardiac remodeling as well as the potential of small mole-
cule stimulators in the reduction of ﬁbrotic tissue.

IL-33 is another member of the IL-1 family of pro-inﬂammatory in-
terleukins. It has long been thought to contribute to ﬁbrosis, in particular
in allergic inﬂammation. This is due to its ability to activate immune cells
such as macrophages, eosinophils, cytotoxic T cells, and group 2 innate
lymphoid cells upon cell damage (Chan et al., 2019; Kondo et al., 2008;
Bonilla et al., 2012). These activated immune cells promote the ﬁbrotic
microenvironment and encourages the subsequent production of further
pro-ﬁbrotic cytokines. The inhibition of IL-33 would theoretically pre-
vent the downstream activation of pro-ﬁbrotic cytokines thus reducing
ﬁbrosis. One study has investigated the role of IL-33 expression in rats
with chronic heart failure (Wang et al., 2017). The IL-33/ST2 signaling
axis was inhibited using the microRNA miR487b, which was found to
reduce the expression of the IL-33 protein, resulting in improved cardiac
morphology and reduced collagen expression in an in vivo model of
chronic heart failure induces by coronary artery occlusion. The use of
microRNAs to inhibit cytokines and the downstream signaling pathways
may therefore be a fruitful avenue to pursue in ongoing efforts to
attenuate tissue ﬁbrosis.

3. TNF-α

TNF-α is produced during inﬂammation by immune cells such as
macrophages and monocytes. TNF-α has various roles within the body,
including pathogen resistance, anti-tumor immunity and sleep regula-
tion. It affects these processes by activating a variety of pathways,
including NF-κB, Erk, MAPK and PLA2 (Idris and Naismith, 2000).
The important roles of TNF-α in regulating inﬂammation explains why it
is also involved in ﬁbrosis as the two often come hand in hand (Fig. 3).

Following organ transplantation, extensive ﬁbrosis often occurs, i.e.
chronic transplant rejection. Zhou et al. (2019) investigated the effect of
a TNF-α inhibitor to prevent EMT, a key driver of interstitial renal
ﬁbrosis. They showed that treatment of HK-2 cells with TNF-α caused an
increase in the EMT markers α-SMA and ﬁbronectin via the activation of
Smurf1, an e3 ubiquitin ligase that has been found to be involved in cell
migration and polarity as well as EMT through its interaction with the
TGF-β canonical pathway and NF-κB signaling (Zhou et al., 2019). The
anti-cancer drug bortezomib, a proteasome inhibitor, has also been
shown to target Smurf1, a HECT-type E3 ubiquitin ligase that plays a
role in disordered tissue remodeling. Treating mice with the small molecule
MCB-613, a steroid receptor coactivator stimulator, was shown to
reduce the phosphorylation of Akt and inhibiting EMT. Moreover,
bortezomib was found to reduce inﬂammation and ﬁbrosis after
orthotopic kidney transplantation in rats (Zhou et al., 2019). This sug-
gests that existing cancer drugs may be useful tools in mitigating fi-
brosis (Wishart et al., 2006).

As previously mentioned, TNF-α can activate the MAPK pathway via
NF-κB, a critical pathway mediating inflammation. In addition, TNF-α is
thought to interact with TLR4 and modulate its expression, with varia-
tions in TLR4 signaling being an important factor in many diseases
(Simonaro et al., 2010). Using the unilateral ureteric obstruction (UUO)
mouse model of renal interstitial fibrosis, Li et al. (2019) tested the
natural drug salidroside and its ability to inhibit the NF-κB pathway.
They showed that salidroside reduced the inflammatory markers TNF-α,
IL-6 and IL-1β in vivo. It also reduced the levels of both collagen I and III
in the ECM and reduced EMT via the downregulation of TGF-β, α-SMA
and vimentin. They postulated that salidroside had these effects by
inhibiting TLR4, which subsequently reduced the expression of the
downstream molecules NF-κB and p-ERK, shown via Western blot (Li
et al., 2019).

Naturally sourced compounds have also been suggested to affect the
TNF-α/NFkB pathway. The drug phillygenin is obtained from the plant
Forsythia suspensa and is often used in traditional Chinese medicine. In an
in vitro study investigating the induction of a fibrotic phenotype in LX-2
hepatic stellate cells, phillygenin reduced the expression of IL-1β, IL-6
and TNF-α, suggesting that it has anti-inflammatory properties (Hu
et al., 2020). Western blot experiments also indicated that treatment with
phillygenin mitigated the induction of fibrosis markers, as it reduced
the expression of α-SMA and collagen 1 (Hu et al., 2020). It was also found
in this study that phillygenin binds directly with TLR4 in order to inhibit the
downstream signaling cascade (Hu et al., 2020). This study highlights the
possibility of using natural drugs to reverse tissue fibrosis and
inflammation.

Periodontal fibrosis has been treated using the flavonoid cynaroside
and the mechanisms behind its activity have been explored (Lee et al.,
2020). The effects of cynaroside were tested in vitro on human peri-
odontal ligament cells. LPS was used as an inductor of fibrosis and

![Fig. 3. TNF-α signaling pathways in fibrosis and the mechanism of action of TNF-α pathway inhibitors.](image-url)
increased the expression of TNF-α, iNOS and COX-2. These increases were reversed with cymarinose, observed via Western blot, and were also observed in RAW264.7 cells. It was hypothesized that this effect was mediated through the inhibition of NF-κB, as cymarinose prevented the translocation of the NF-κB p65 subunit to the nucleus. This prevent the resulting signalling cascade and impaired the induction of fibrosis. It was also shown that treatment with cymarinose encouraged the differentiation of periodontal ligament cells into a more osteogenic morphology, so this flavonoid may be useful for repairing damaged teeth (Lee et al., 2020). By preventing the action of TNF-α through the arrest of NF-κB as well as reforming bone tissue, cymarinose is a promising treatment for periodontal fibrosis.

Chronic respiratory disease often involves severe inflammation and, in chickens, can be caused by mycoplasma infection. zou et al. (2020) proposed using polydatin, a resveratrol glycoside isolated from Polygornum cuspidatum, to treat this inflammation. Damaged lungs showed increased numbers of inflammatory cells, shedding of epithelial cells and alveolar congestion. These changes were not present in lungs treated with polydatin, suggesting a molecular intervention with this anti-fibrotic compound. Polydatin was also shown to reduce TLR6 expression in a dose-dependent manner. TLR6 activates the NF-κB pathway, with Zou showing that polydatin treatment resulted in a decrease in nuclear NF-κB translocation. Proinflammatory cytokines, including TNF-α, were also markedly decreased following polydatin treatment, suggesting that polydatin successfully blocks the effect of proinflammatory cytokines (Zou et al., 2020). Upstream targeting by polydatin allows the inhibition of a variety of cytokines and thus further reduces the impact of inflammation on tissue morphology.

Opuntioil, the active ingredient from Opuntia ficus-indica, is known for its anti-UV properties and is being investigated as a treatment against UVA-induced skin fibrosis (Kandan et al., 2020). Overall, opuntioil reduced the clinical score of fibrosis severity in mice following UVA exposure. In addition, pretreatment with opuntioil prevented epidermal hyperplasia and loss of collagen. This suggests that opuntioil has a molecular protective effect which results in the prevention of UV damage. Using Western blot, the location of NF-κB and AP-1 was determined, demonstrating that opuntioil prevented the nuclear translocation of NF-κB and AP-1, thereby preventing transcription. With immunohistochemistry, it was highlighted that topical treatment with opuntioil prevented the build-up of inflammatory proteins such as TNF-α, COX-2 and iNOS. This suggests that the blockade of the NF-κB prevented TNF-α-mediated inflammation from further exacerbating fibrosis (Kandan et al., 2020). The effect of UV on pro-inflammatory cytokines was effectively prevented by opuntioil, suggesting that it may be a good topical treatment for UV-induced fibrosis.

Son et al. highlighted the positive effects of paclitaxel on diabetic nephropathy via targeting TNF-α and TLR4 (Son et al., 2020). An in vitro study was carried out using podocytes to simulate diabetic nephropathy. Podocyte injury induced by palmitate exposure increased the mRNA levels of TNF-α and TLR4, but this increase was abrogated by treatment with paclitaxel. Along with this, reactive oxygen species and NOX4, which controls reactive oxygen species production, were increased after injury and reduced following treatment. This intervention may have contribute to a reduction in podocyte damage, as paclitaxel treatment prevented F-actin rearrangement, reduced VEGF mRNA levels and increased nephrin expression (Son et al., 2020).

N-acetyl cysteine is thought to inhibit the formation of ROS, but has been suggested to also have an anti-fibrotic effect. In a study by Honma et al., renal interstitial fibrosis was simulated in mice in order to investigate the effect of N-acetyl cysteine on renal fibrosis (Honma et al., 2020). The overexpression of TNF-α observed in the fibrotic kidney was attenuated following N-acetyl cysteine treatment, along with a reduction in the mRNA levels of fibrosis-associated collagen III. The MAPK pathway was also affected by N-acetyl cysteine, as this intervention reduced ERK1/2 phosphorylation, although the JNK and p38 pathways were not affected. Honma et al. concluded that the intervention with N-acetyl cysteine contributed to a reduction in TNF-α and subsequent reduction in collagen production (Honma et al., 2020). As ERK1/2 is a member of several cytokine pathways, this downstream intervention may be key to effectively combating fibrosis.

Clinical trials have been undertaken using the TNF-α inhibitor etanercept in order to treat cytotoxic T lymphocyte-mediated severe cutaneous adverse reactions (SCARs) (Wang et al., 2018). It was hypothesized that TNF-α was responsible for the heightened immune response in SCARs as well as the death of keratinocytes. As etanercept is a TNF-α antagonist, it was able to reduce the levels of TNF-α as well as the cell death mediator granulysin both in vitro and in vivo. In human trials, Wang et al. also demonstrated that etanercept treatment caused skin lesions to heal faster and with less inflammation than with standard corticosteroid treatment. They also showed that etanercept treatment increased the population of regulatory T cells in the lesion, which also reduced patient mortality (Wang et al., 2018). The ability of TNF-α to modulate the immune system under inflammatory and fibrotic conditions highlights the possibility of targeting TNF-α for anti-inflammatory and anti-fibrotic therapies.

4. IL-6

One of the downstream mediators of the aforementioned IL-1 family is the inflammatory interleukin IL-6 (Fig. 4), which plays an active role in the modulation and activation of the immune response; defects in IL-6 signaling play a role in many inflammatory and autoimmune diseases (Hirano, 2010). IL-6 activates the JAK/STAT3 pathway and the SHP2/Gab/MAPK pathway and interacts with the NLRP3 inflammasome (Hirano, 2010; Del Campo et al., 2018). Its ability to induce monocyte differentiation into macrophages and activate Th2 and Th17 immune cells also indicates its influence on inflammation and fibrosis (Su et al., 2017; Fielding et al., 2014). The activation of Th2 and Th17 can be explored using different models of fibrosis, with more mild fibrosis being Th2-driven, whilst severe fibrosis is characterized by Th17 responses, more often seen in unresolved, chronic infections and inflammatory diseases (Zhang and Zhang, 2020). For example, the house dust mite model of allergic airway disease induces a mainly Th2 immune response, but extended aeroallergen exposure leads to a more Th17-skewed response with extensive tissue remodeling characterized by EMT (Johnson et al., 2004, 2011, 2015).

With the signaling pathways of IL-6 being so varied, there have been several different methods of intervention when therapeutically targeting IL-6. A study by Chou et al. (2018) investigated the effects of IL-6 in aldosterone-induced cardiac fibrosis. They used a natural existing inhibitor of IL-6, sgp130, which can block IL-6 and its soluble form from binding and inducing the trans-signaling pathway in endothelial cells. By analyzing the expression of fibronectin and collagen-1, they suggested that this inhibitor mitigates fibrosis promoted by IL-6. In vivo, they also showed that recombinant gp130 decreased cardiac fibrosis by observing reduced ventricular fibrosis following aldosterone infusion in mice (Chou et al., 2018). This study highlighted the impact of IL-6 on fibrosis and the potential for therapeutics that interrupt the IL-6 trans-signaling pathway.

Fatty liver disease is another fibrotic condition that is exacerbated by IL-6. In rats with fatty liver disease, serum levels of IL-6 increased significantly, but were decreased following lycopene treatment (Saeeda et al., 2020). Similarly, NF-κB levels were increased in diseased tissue and decreased with lycopene. Lycopene treatment also prevented structural changes such as increased α-SMA expression. Further staining also concluded that lycopene reduced the number in inflammatory cells present in the effected tissue and mitigated collagen deposition. Saeed et al. concluded that IL-6 levels are an indicator of insulin resistance and liver damage, and that a reduction in circulating IL-6 leads to a reduction in NF-κB activation and therefore a reduction in structural remodeling (Saeeda et al., 2020). With lycopene also targeting insulin resistance as well as pro-inflammatory cytokine signaling, this two-pronged approach may be an effective treatment for fatty liver diseases and associated...
renal fibrosis.
Renal fibrosis is also affected by IL-6 signaling and studies have identified a tocilizumab mimotope that can target the IL-6 receptor in order to suppress this signaling pathway (Yang et al., 2020). Via a vaccine strategy, the mimotope was administered to mice with renal fibrosis established using the UUO model in order induce inhibitory antibodies that target IL-6R. Mimotope treatment decreased levels of fibronectin, collagen and α-SMA compared to untreated diseased tissue via the modulation of IL-6 signaling. This treatment also suppressed the differentiation of macrophages in the kidneys, further reducing the secretion of pro-inflammatory cytokines. Vaccination also had downstream effects, as it reduced the phosphorylation of ERK, thereby preventing that pathway from contributing to fibrosis. Further injury to the kidney could also be at least partly prevented by the tocilizumab mimotope, as it slightly reduced the levels of ferroptosis, which leads to additional kidney injury. Although the mimotopes successfully inhibited pERK, no change was observed in the levels of phosphorylated STAT3, so only part of the IL-6 pathway was successfully inhibited (Yang et al., 2020). Despite this, the effects of the tocilizumab mimotope showed promising effects in terms of preventing kidney fibrosis.

5. TGF-β

It is widely recognized that TGF-β is one of the main cytokines involved in fibrosis (Fig. 5). It is upregulated in most fibrotic diseases; however, paradoxically, it also has anti-inflammatory effects. This is due to two major signaling pathways, i.e. the canonical pathway mediated by a cascade of Smad protein interactions and the non-canonical pathway with activation of mTOR, Erk, JNK and p38 signaling. However, due to its widespread roles in proliferation, differentiation and various other homeostatic functions, inhibiting TGF-β upstream may induce many side effects. Therefore, any drugs targeting TGF-β are likely to instead target the Smad signaling pathway (Kubiczkova et al., 2012; Biernacka et al., 2011; Meng et al., 2016).
A study by Yang et al. isolated the natural TGF-β inhibitor isorhamnetin from the leaves of the plant *Oenanthe javanica* (Yang et al., 2016). They showed that treatment with 50–100 μM isorhamnetin reduced the expression of fibrotic markers α-SMA and PAI-1 in TGF-β-treated hepatic stellate cells. Isorhamnetin also decreased Smad binding element activity and greatly inhibited the phosphorylation of Smad2 and Smad3, suggesting that it targets this stage of the canonical TGF-β signaling cascade. They also highlighted the protective effect of isorhamnetin against oxidative stress by preventing TGF-β-induced reactive oxygen species production and by activating the antioxidant protein Nrf2 (Yang et al., 2016). This two-pronged approach is promising as it combats two triggers of fibrosis whilst not blocking TGF-β activity completely, which should reduce possible side effects.

Another study on the role of TGF-β in liver fibrosis utilized a mouse model to investigate the relationship between TGF-β and another member of the TGF family, BMP7 (Zou et al., 2019). These authors observed that mice with induced liver fibrosis had upregulated levels of TGF-β and Smad3, and downregulated BMP7 and Smad1/5/8. This suggests that Smad1/5/8 signaling might inhibit the fibrotic effects of TGF-β via BMP7 (Guo et al., 2017). They also showed that, in vitro, a high dose BMP7 delivered to TGF-β-treated hepatic stellate cells reduced the production of the myofibroblast markers α-SMA and collagen I and moreover inhibited p38-induced migration and proliferation. This suggests that BMP7, via Smad1/5/8, inhibits hepatic stellate cell activation and mitigates fibrosis through a reduction in activated myofibroblasts (Zou et al., 2019). The use of exogenous proteins and existing inhibitory signaling pathways could provide an alternative for treating liver fibrosis.

Fig. 5. TGF-β signaling pathways in fibrosis and the mechanism of action of TGF-β pathway inhibitors.
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indicating that TGF-β mediate modulation of the extracellular matrix enzymes MMP-2 and MMP-9, which is often used as a marker for renal inflammation. Using Western blot, Yi et al. showed that metformin inhibited the phosphorylation of Smad3 as well as ERK1/2 and p38, thus suppressing both the canonical and non-canonical TGF-β signaling pathways (Yi et al., 2021). By targeting both arms of the TGF-β signaling pathway, metformin may have a considerable effect in terms of mitigating fibrosis.

6. Outlook

There are many different types of cytokine inhibitors currently being investigated as potential treatments for fibrosis in various organs. The variety of strategies and targets discussed here indicates the scope of this area of research and the complexity of the systems at play in fibrosis. Some treatments do not directly inhibit the target cytokine and instead target a molecule upstream (in the case of emecasan and seleralaxin), thereby preventing the active cytokine from being produced, or downstream mediators (e.g. bortezomib and salidroside) to prevent the signaling cascade and the action of the cytokine from occurring in full. There is also a possibility of harnessing the body’s innate defenses, such as in the case of IL-1Ra and BMP7, and either increasing or modulating their effects in order to combat fibrosis. There is also an increasing number of natural, plant-based compounds being explored for their anti-fibrotic properties, including phillygenin, lycopene and isohamnetin, which have been used in herbalism and traditional medicine systems and have been more recently shown to have scientific promise in the case of treating fibrosis. Despite the complexity of the interplay of cytokines in fibrotic tissues, the breadth of the currently ongoing research targeting cytokines suggests that these may hold the key to mitigating tissue fibrosis and reducing organ damage in the future.

CRediT authorship contribution statement

Rebecca Bignold: performed the systematic review and prepared the manuscript. Jill R. Johnson: Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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