



## Review

## Diabetic fibrosis

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## ABSTRACT

Diabetes-associated morbidity and mortality is predominantly due to complications of the disease that may cause debilitating conditions, such as heart and renal failure, hepatic insufficiency, retinopathy or peripheral neuropathy. Fibrosis, the excessive and inappropriate deposition of extracellular matrix in various tissues, is commonly found in patients with advanced type 1 or type 2 diabetes, and may contribute to organ dysfunction. Hyperglycemia, lipotoxic injury and insulin resistance activate a fibrotic response, not only through direct stimulation of matrix synthesis by fibroblasts, but also by promoting a fibrogenic phenotype in immune and vascular cells, and possibly also by triggering epithelial and endothelial cell conversion to a fibroblast-like phenotype. High glucose stimulates several fibrogenic pathways, triggering reactive oxygen species generation, stimulating neurohumoral responses, activating growth factor cascades (such as TGF- $\beta$ /Smad3 and PDGFs), inducing pro-inflammatory cytokines and chemokines, generating advanced glycation end-products (AGEs) and stimulating the AGE-RAGE axis, and upregulating fibrogenic matricellular proteins. Although diabetes-activated fibrogenic signaling has common characteristics in various tissues, some organs, such as the heart, kidney and liver develop more pronounced and clinically significant fibrosis. This review manuscript summarizes current knowledge on the cellular and molecular pathways involved in diabetic fibrosis, discussing the fundamental links between metabolic perturbations and fibrogenic activation, the basis for organ-specific differences, and the promises and challenges of anti-fibrotic therapies for diabetic patients.

## 1. Introduction

Estimates by the International Diabetes Federation and the World Health Organization suggest that diabetes affects more than 400 million individuals worldwide [1]. Due to the rapidly increasing prevalence of obesity, which predisposes for type 2 diabetes, the number of diabetics is expected to markedly increase over the next 20 years. Most of the global burden of diabetes is due to morbidity and mortality that arises from complications of the disease [2]. Diabetics exhibit an increased incidence of several chronic debilitating conditions, including chronic renal insufficiency, heart failure, hepatic insufficiency, retinopathy and peripheral neuropathy. These conditions are major causes of morbidity and mortality in diabetics.

Fibrosis, the excessive and/or inappropriate deposition of extracellular matrix (ECM) proteins, is often found in diabetic tissues and may account for organ dysfunction. Although in some cases, diabetes-associated fibrosis may represent an epiphenomenon, reflecting repair of primary injury, a large body of evidence supports the notion that the

metabolic dysregulation observed in diabetic subjects may directly activate a fibrogenic program, causing tissue injury and organ dysfunction. The pro-fibrotic effects of diabetes may involve direct activation of resident fibroblasts by hyperglycemia or insulin resistance, or stimulation of a fibrogenic program in immune cells, vascular cells, or organ-specific parenchymal cells. The current review manuscript focuses on the cell biological basis and the molecular mechanisms of fibrosis in diabetic subjects. Moreover, we discuss the basis for organ-specific patterns of fibrogenic activation in diabetics, the functional consequences of fibrosis in various organs and the promises and challenges of anti-fibrotic interventions in diabetic subjects.

## 2. Fibrotic changes in diabetic tissues

In both animal models of diabetes and in diabetic patients, organ dysfunction is typically associated with fibrotic changes. Fibrosis has been suggested to contribute to the pathogenesis of diabetic nephropathy, cardiomyopathy and liver dysfunction and may also be involved in

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the development of diabetic retinopathy and neuropathy. Patients with longstanding diabetes often exhibit extensive fibrotic lesions in the heart, kidney or liver. However, to what extent these fibrotic changes represent direct effects of metabolic dysfunction, or reflect complications of atherosclerotic disease, or the high prevalence of other pro-fibrotic comorbid conditions, such as hypertension and dyslipidemias, remains unknown. Thus, most of our information on the direct fibrogenic effects of diabetes and metabolic dysfunction is derived from experimental studies using animal models of type 1 and type 2 diabetes (Table 1). Although these models recapitulate important aspects of human diabetic disease, such as hyperglycemia or insulin resistance, and are generally associated with fibrotic changes in several different organs, they also have significant limitations. First, fibrotic changes in rodent models of diabetes are generally mild (Fig. 1), in comparison to the much more extensive fibrosis noted in patients with long-standing diabetes. This may reflect the absence of significant atherosclerotic disease in rodents that may be a relevant contributor in cardiac or renal fibrotic remodeling in patients. Second, in chemically-induced models of type 1 diabetes, the agents used to target  $\beta$ -cells may exert injurious actions on other cell types, thus limiting conclusions regarding the effects of hyperglycemia. Third, genetic models of diabetes may exhibit unique abnormalities related to the underlying mutation that may complicate interpretation of the findings, limiting their translatability to the human disease process. On the other hand, diet-induced models of insulin resistance and type 2 diabetes may be more relevant to the human pathophysiology, but typically require long periods of follow-up to develop fibrotic changes, and may exhibit highly variable responses, depending on the specific characteristics of the diet used, the age, genetic background and sex of the animals [3,4]. Ultimately, no animal model can fully recapitulate the complexity of human disease. Thus, understanding the mechanisms involved in diabetes-associated fibrosis cannot rely on single studies using a specific animal model, but requires consideration of several different *in vivo* models, *in vitro* experiments and clinical investigations.

### 3. The cellular effectors of diabetic fibrosis

Although fibroblasts are the main source of ECM proteins in fibrotic conditions, other cell types may contribute to fibrotic responses by secreting growth factors and cytokines that activate fibroblast phenotype and function. Moreover, it has been suggested that when stimulated by fibrogenic growth factors, several cell types (including epithelial cells, endothelial cells, vascular mural cells, and subsets of circulating progenitors) may acquire a fibroblast phenotype, directly contributing to matrix synthesis and deposition. Ample evidence suggests that diabetes-associated metabolic dysregulation directly activates a fibrogenic program in several different cell types. Moreover, diabetes-associated fibrosis may also reflect the indirect activation of reparative fibroblasts, in response to the injurious effects of hyperglycemia, insulin resistance and metabolic dysfunction on organ-specific parenchymal cells, such as cardiomyocytes or hepatocytes.

#### 3.1. Activation of fibroblasts and myofibroblasts in diabetic tissues

Fibroblast to myofibroblast conversion is a central event associated with activation of a matrix-synthetic program in healing and fibrotic tissues. In response to neurohumoral activation, growth factor stimulation, or mechanical stress, fibroblasts become contractile and secretory, acquiring stress fibers decorated with  $\alpha$ -smooth muscle actin and producing large amounts of matrix proteins. These activated myofibroblasts are the main matrix-producing proliferative cells in healing wounds and in most fibrotic conditions [5–7]. In normal wound healing myofibroblasts eventually become quiescent, acquiring a unique profile of matrix protein expression that serves to preserve the scar in non-regenerative organs, such as the myocardium [7]. Although fibroblast activation and increased matrix-synthetic capacity are consistently noted in

**Table 1**

Fibrosis in rodent models of type 1 and type 2 diabetes.

Model of diabetes	Mechanism	Characteristics of fibrotic response
Type 1 diabetes		
Chemically-induced type 1 diabetes through injection of toxic glucose analogs, such as streptozotocin (STZ) or alloxan	STZ or alloxan injection results in selective uptake of STZ by pancreatic $\beta$ -cells via the glucose 2 transporter (GLUT2), and subsequent cytotoxicity, culminating in cell death and development of diabetes due to absence of insulin. Alloxan also selectively attenuates glucose-induced insulin secretion by inhibiting the $\beta$ -cell glucose sensor glucokinase [293].	<ul style="list-style-type: none"> <li>STZ-induced diabetic mice have been reported to develop cardiac [294] and renal [295] fibrosis.</li> <li>Effects seem to be dependent on strain: STZ-induced diabetic CD1 mice develop more prominent renal fibrosis and dysfunction than C57BL/6 or 129Sv mice [296].</li> <li>Direct effects of STZ on other cell types (such as hepatocytes and renal tubular cells) may somewhat limit the value of the model.</li> <li>Fibrotic changes in alloxan-induced models are less characterized; however, hepatic fibrosis has been reported [297].</li> <li>OVE26 mice develop severe nephropathy, associated with albuminuria and glomerular and tubulointerstitial fibrosis [299] and also exhibit cardiac fibrosis [300].</li> <li>Strain is an important determinant of disease severity: FVB background is associated with more severe fibrotic changes than C57BL/6 [301].</li> <li>DP-BB rats exhibit renal fibrosis, associated with activation of the TGF-<math>\beta</math> axis [302].</li> </ul>
OVE26 transgenic mouse	OVE26 mice overexpress calmodulin in pancreatic $\beta$ -cells leading to $\beta$ cell injury and severe early onset type 1 diabetes [298].	
Diabetes-prone biobreeding (DP-BB) rat	DP-BB rats exhibit a spontaneous mutation, leading to autoimmune $\beta$ -cell destruction and type 1 diabetes	
Akita (Ins2 <sup>+/-</sup> ) mouse	Akita mice have a spontaneous mutation in the <i>insulin 2</i> gene preventing pro-insulin processing, and leading to overload of misfolded proteins and endoplasmic reticulum (ER) stress.	<ul style="list-style-type: none"> <li>Akita mice were found to exhibit renal glomerular and tubulointerstitial fibrosis [303]. Myocardial fibrotic changes were noted in some studies [304], but not in others, despite the early development of diastolic dysfunction [305].</li> <li>Genetic background, sex-specific effects and distinct dietary protocols may explain the conflicting findings.</li> </ul>
Type 2 diabetes		
Leptin-resistant db/db mouse	The db/db mouse has a point mutation in the gene encoding the leptin receptor, resulting in a protein with a truncated cytoplasmic domain that is functionally inactive [306]. As a result, db/db mice are resistant to the central effects of leptin (but may exhibit leptin	<ul style="list-style-type: none"> <li>The best-studied genetic model of type 2 diabetes.</li> <li>db/db mice exhibit renal fibrosis [307], and cardiac fibrosis [12].</li> <li>The severity of fibrotic changes is dependent on sex, age, genetic background and diet of the animals studied.</li> <li>Signaling through the truncated leptin receptor may complicate</li> </ul>

(continued on next page)

Table 1 (continued)

Model of diabetes	Mechanism	Characteristics of fibrotic response
Leptin-deficient ob/ob mouse	responses in peripheral cells that express the truncated leptin receptor), and develop hyperphagia, severe obesity and overt diabetes. Leptin-deficient mice develop hyperphagia, severe obesity and diabetes.	interpretation of the findings. <ul style="list-style-type: none"><li>• When placed on the BTBR (black and tan brachyuric) background, leptin-deficient ob/ob mice develop significant renal fibrosis [308].</li><li>• In contrast, in a C57Bl/6 background, ob/ob mice have mild metabolic changes and do not develop significant cardiac fibrosis [309].</li></ul>
Zucker obese rat	Zucker rats have a leptin receptor missense mutation, and develop hyperphagia, obesity and insulin resistance.	<ul style="list-style-type: none"><li>• Zucker rats develop myocardial fibrosis [310], hepatic fibrosis accompanied by NASH [311], and renal fibrosis that may precede hyperglycemia [312].</li></ul>
Goto-Kakizaki (GK) rat (polygenic non-obese)	Spontaneously diabetic rat strain with impaired glucose tolerance in the absence of obesity.	<ul style="list-style-type: none"><li>• GK rats exhibit pancreatic islet fibrosis [313] and late onset of renal [314] and cardiac fibrosis [315].</li></ul>
Otsuka Long-Evans Tokushima Fat (OLETF) rat, (polygenic obese)	OLETF rats exhibit mild obesity and insulin resistance followed by late onset of hyperglycemia.	<ul style="list-style-type: none"><li>• OLETF rats exhibit renal fibrosis [316] and cardiac perivascular and interstitial fibrosis that may precede development of diabetes [317].</li></ul>
Diet-induced models of insulin resistance	Administration of high-fat diets or high-fat high-carbohydrate diets induces obesity and insulin resistance.	<ul style="list-style-type: none"><li>• High-fat diet-induced insulin resistance promotes fibrosis in many organs, including the heart and the kidney.</li><li>• The severity and time course of fibrotic remodeling are dependent on the type of the diet, the sex and age of the mice, and their genetic characteristics.</li><li>• In many studies, fibrosis is mild and requires prolonged feeding [3].</li><li>• High-fat/high-sucrose or high-fat/ high-fructose diets may worsen metabolic profile and augment the fibrogenic actions [20,318].</li></ul>
Combination of low-dose STZ and diet-induced diabetes	This model recapitulates advanced stages of type 2 diabetes in humans characterized by insulin resistance and $\beta$ -cell failure.	<ul style="list-style-type: none"><li>• In rats, low-dose STZ combined with a high-fat/high-fructose diet induced fibrotic changes in several organs, including the kidney, liver and heart [319].</li><li>• Severity of fibrosis may be dependent on the genetic background and sex of the rodents, and on the type of diet used (high-fat vs. high-fat/high-sucrose vs. high-fat/high-fructose)</li></ul>

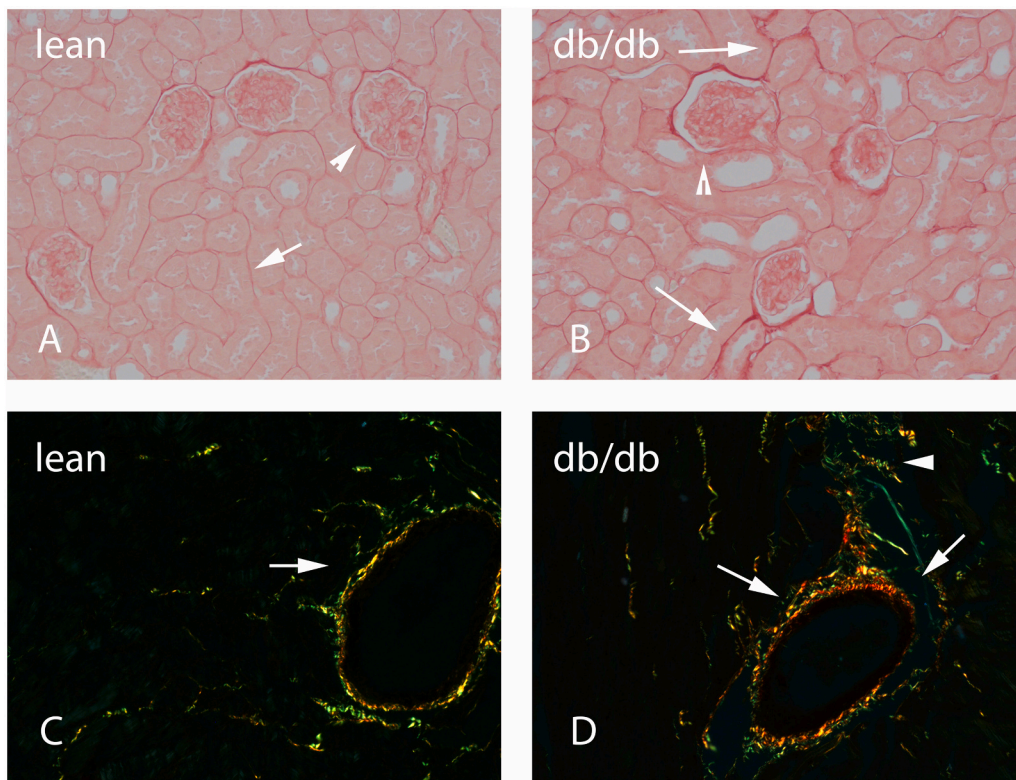
diabetic tissues, to what extent myofibroblast conversion is responsible for diabetes-associated fibrotic remodeling remains unknown. In vitro experiments have demonstrated that glycated collagen can activate fibroblast to myofibroblast conversion [8] and in vivo studies have reported increased density of myofibroblasts in rodent models of diabetic renal disease [9–11]. In contrast, other studies suggested that myofibroblast conversion may not be required for fibrotic remodeling in diabetic tissues. In obese diabetic db/db mice, increased myocardial collagen deposition and expansion of the collagen-producing population of cardiac interstitial cells occurred in the absence of myofibroblast conversion [12]. These findings suggest that diabetic fibrosis may involve alternative pathways of fibroblast activation. Diabetes-associated alterations in fibroblast phenotype may have common characteristics with the changes observed in aging tissues, in which fibrosis and activation of interstitial cells is associated with perturbed myofibroblast conversion [13,14]. Moreover, systematic transcriptomic analysis of fibroblast populations in reparative and fibrotic conditions has identified populations of activated fibroblasts that may lack myofibroblast characteristics, but exhibit enhanced matrix synthetic capacity [15–17]. Single cell transcriptomic studies are needed to identify fibroblast subsets that may be selectively activated in diabetic subjects, driving the fibrogenic response.

The origin of activated fibroblasts in diabetic tissues is controversial and is likely dependent on the organ affected and on contextual factors. Most organs contain populations of fibroblast-like cells that serve to preserve baseline structure, while acting as reparative cells following injury [18,19]. Inappropriate, excessive or prolonged activation of resident fibroblasts may promote matrix deposition, stimulating tissue fibrosis and causing organ dysfunction. In diabetes, metabolic dysregulation may stimulate activation and expansion of resident fibroblast populations in several different organs. Several studies have concluded that in addition to resident fibroblasts, several other cell types may contribute to diabetes-associated fibroblast expansion. It has been suggested that inappropriate activation of endothelial cells (through endothelial to mesenchymal transition/EndMT), epithelial cells (through epithelial to mesenchymal transition/EMT), pericytes, or circulating hematopoietic cells may significantly contribute to fibroblast populations in diabetic organs [20–22]. Most of the evidence on the origin of fibroblasts involved in diabetic fibrosis is based on associative data showing co-expression of fibroblast markers and proteins considered specific for vascular or hematopoietic cells. Lineage tracing data are limited. In a mouse model of type 1 diabetes, lineage tracing of endothelial cells with the Tie2-Cre driver suggested that a significant fraction (23%) of renal interstitial myofibroblasts were of endothelial origin [23]. EndMT in the diabetic heart was attributed to activation of the Transforming Growth Factor (TGF)- $\beta$ /Smad3 pathway in endothelial cells [24]. Unfortunately, the lack of specificity of the Tie2 Cre driver for endothelial cells and the challenges in reliable identification of fibroblasts limit the conclusions of these studies. Thus, the relative contribution of vascular endothelial cells in expansion of the fibroblast population in diabetic tissues remains unclear.

### 3.2. Macrophages, mast cells and lymphocytes

Macrophages can produce a wide range of potent fibrogenic mediators (including TGF- $\beta$ s and Platelet-derived Growth Factors (PDGFs)), thus stimulating fibroblast activation [25,26]. Several studies have documented macrophage recruitment and activation in experimental models of diabetes, and have demonstrated that macrophage accumulation is associated with tissue fibrosis and organ dysfunction [27–29]. Recruitment of macrophages in diabetic tissues may involve hyperglycemia-mediated induction of adhesion molecules, such as Interleukin Adhesion Molecule (ICAM)-1 [30], and release of monocyte chemoattractant chemokines, such as CCL2 [31]. In db/db mice, global loss of ICAM-1 or CCL2 attenuated renal fibrosis and protected the kidneys from dysfunction, in the absence of effects on obesity or





**Fig. 1.** Fibrotic changes in animal models of diabetes. Obese diabetic leptin-resistant db/db mice exhibit fibrotic changes in several different organs, including the kidney (A-B) and the heart (C-D). A-B: Representative images from kidney sections from a lean (A) and a db/db mouse at 6 months of age stained with PicroSirius red to label collagen fibers. Please note the mild fibrosis involving the glomerulus (arrowhead) and the tubulointerstitium (arrows) in db/db mice. C-D: Representative images from left ventricular myocardial sections from a lean (C) and a db/db mouse (D) at 6 months of age, stained with PicroSirius red and visualized under polarized microscopy. The db/db mouse shows modest expansion of the collagen network in perivascular (arrows) and interstitial areas (arrowheads). In contrast to the extensive fibrosis noted in human diabetic patients, changes in rodent models of diabetes are generally mild. This likely reflects the absence of atherosclerotic disease and other comorbid conditions that often accompany diabetes in human patients, and the young age of the experimental animals.

metabolic dysfunction. The protective effects of ICAM-1 and CCL2 loss were attributed to reduced local recruitment and activation of macrophages. Inhibition of interactions between the crucial macrophage growth factor Colony Stimulating Factor (CSF)-1 and its receptor c-fms also attenuated renal fibrosis and dysfunction in obese diabetic db/db mice [32], further supporting the notion that macrophages may be involved in fibrosis of the diabetic kidney.

Mast cells can also produce and secrete a wide range of fibrogenic mediators, including growth factors and cytokines (such as TGF- $\beta$  and Tumor Necrosis Factor (TNF)- $\alpha$ ), chymase (known to convert angiotensin I to angiotensin II), tryptase and matrix metalloproteinases (MMPs). Hyperglycemic conditions may directly activate mast cells contributing to release of fibrogenic granular contents [33]. Increased density of myocardial mast cells has been reported in a rodent model of type 1 diabetes [34], and mast cell deficiency attenuated both ventricular and atrial diabetic myocardial fibrosis [35,36].

Lymphocytes have also been implicated in the pathogenesis of fibrotic conditions in several different organs, and may also participate in regulation of the diabetic fibrotic response. In diabetic animals, sustained low-level inflammation is associated with induction of endothelial cell adhesion molecules and chemotactic cytokines and chemokines, promoting recruitment of T lymphocytes [37]. It has been suggested that chronic hyperglycemia in diabetic subjects may activate T cells through RAGE-mediated epigenetic mechanisms, inducing expression of Th1, Th2 and Th17 cytokines [38]. Lymphocyte-derived Th2 cytokines (such as IL-4, IL-10 and IL-13) are potent fibrogenic mediators and may subsequently activate fibroblasts, contributing to the pathogenesis of diabetic fibrosis. Our knowledge on the potential role of specific lymphocyte subpopulations in diabetes-associated fibrogenic responses remains limited. Antibody neutralization experiments in a model of type 2 diabetes showed that the Th17 cytokine IL-17A is implicated in the pathogenesis of albuminuria and renal fibrosis [39]. In non-alcoholic steatohepatitis (NASH), a major complication of diabetes that is often associated with fibrosis, perturbations in fatty acid metabolism have been implicated in modulation of T lymphocyte profile [40,41]. In the

majority of NASH patients B and T lymphocytes form aggregates that positively correlate with fibrotic changes [42]. B2 lymphocyte-mediated activation of CD4+ T cells [42], natural killer T cells [43] and CD8+ T cells [44] have been implicated in progression of NASH towards fibrosis, and may act by stimulating hepatic stellate cells.

### 3.3. Vascular endothelial and mural cells

Vascular cells, including endothelial cells, pericytes and vascular smooth muscle cells (VSMCs) can contribute to diabetes-associated fibrosis by undergoing conversion to matrix-producing fibroblast-like cells, or by secreting fibrogenic mediators that stimulate fibroblast activation. In diabetics, fibrogenic activation of vascular cells may have broad systemic effects by promoting aortic fibrosis, thus increasing vascular stiffness and exacerbating arterial hypertension. Studies using mice with endothelial cell-specific mineralocorticoid receptor loss showed that neurohumoral activation of endothelial cells plays a critical role in development of aortic fibrosis in a model of high fat diet-induced metabolic dysfunction [20]. Fibrogenic actions of endothelial cells may be mediated, at least in part through secretion of endothelin (ET)-1 [21].

Although vascular mural cells can undergo fibroblast conversion, or acquire a matrix-synthetic phenotype, thus contributing to the pathogenesis of fibrosis [45,46], their involvement in diabetic fibrotic remodeling is poorly documented. Overexpression of the transcription factor Runx2 in VSMCs increased aortic fibrosis and stiffness in the db/db mouse model of type 2 diabetes [47]. Whether hyperglycemia and insulin resistance can activate a pro-fibrotic phenotype in mural cells remains unknown.

## 4. Molecular mechanisms of diabetic fibrosis

Hyperglycemia and insulin resistance are the fundamental metabolic perturbations that trigger fibrogenic cascades in diabetic subjects. Their pro-fibrotic actions are mediated through direct effects on fibroblasts, but also via fibrogenic activation of immune cells, microvascular cells

and epithelial cells, and through indirect mechanisms involving injurious actions on organ-specific parenchymal cells (such as hepatocytes or cardiomyocytes), which are sensed by reparative fibroblasts, stimulating matrix deposition.

#### 4.1. The fibrogenic effects of hyperglycemia

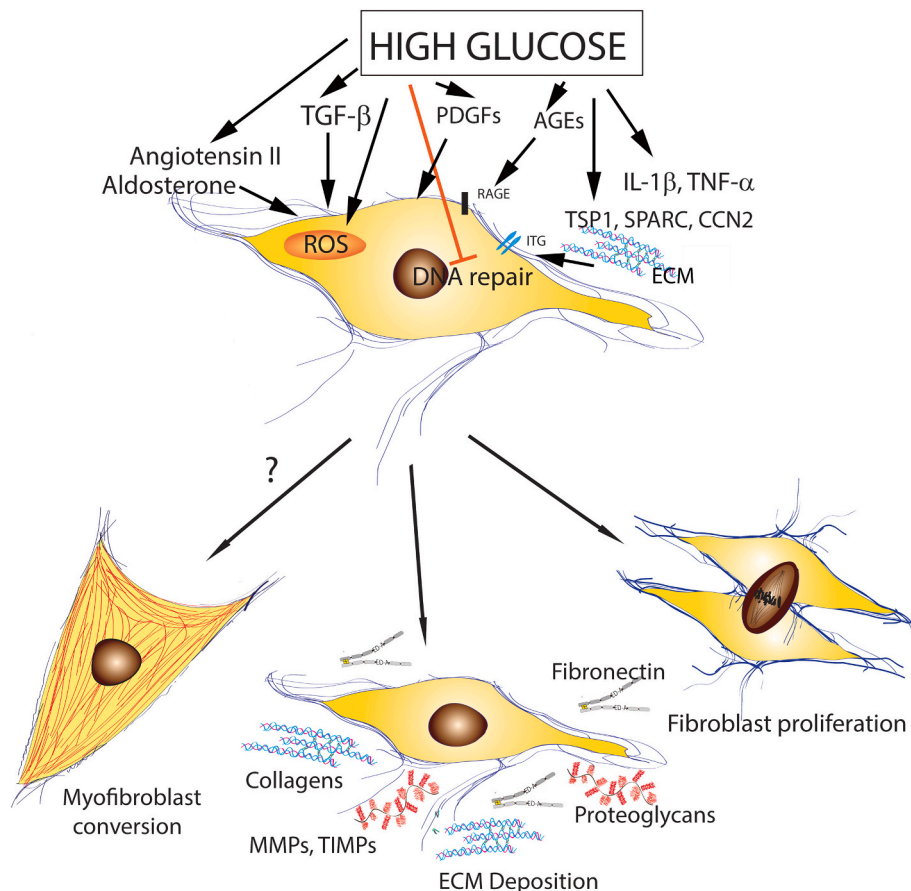
Several lines of evidence support the notion that hyperglycemia is a significant contributor in diabetes-associated fibrosis. First, *in vitro* studies have demonstrated that exposure of tissue fibroblasts to a high glucose environment induces synthesis of fibrillar [48] and non-fibrillar collagens [49], and of specialized ECM proteins, such as fibronectin and matricellular macromolecules [50]. Second, in animal models, administration of anti-hyperglycemic medications markedly attenuates fibrotic changes [51]. Third, in human patients, worse glycemic control is typically associated with evidence of organ fibrosis [52,53]. However, it should be emphasized that experimental and clinical studies have suggested that tight glycemic control may not be sufficient to completely abrogate fibrosis [54], thus suggesting that additional mechanisms may be involved in diabetes-associated fibrotic remodeling. Hyperglycemia-mediated fibrosis involves activation of several fibrogenic pathways, including oxidative stress, neurohumoral signaling, pro-inflammatory cascades, and growth factor-mediated responses (Fig. 2).

##### 4.1.1. The hyperglycemia/reactive oxygen species (ROS) axis

Enhanced ROS generation is a central event in cells exposed to high glucose and may play an important role in fibroblast activation. Intracellular glucose transport and oxidation stimulate mitochondrial superoxide production, leading to inhibition of the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and subsequent accumulation of early glycolytic intermediates [55]. These early

glycolytic intermediates are shunted into pathogenic signaling pathways that cause cell dysfunction, inflammatory activation and fibrosis [56]. Excess of glucose activates aldose reductase, which reduces glucose to sorbitol by using nicotinamide adenine dinucleotide phosphate (NADPH) in the polyol pathway. Resultant NADPH insufficiency impairs reduction of glutathione, perturbing a key antioxidant mechanism and accentuating oxidative stress, thus increasing fibroblast activation. Fructose-6-phosphate may be converted into glucosamine-6 phosphate through glutamine fructose-6 phosphate amidotransferase (GFAT), which then generates uridine diphosphate (UDP) *N*-acetyl glucosamine, inducing synthesis of pro-fibrotic mediators, such as TGF- $\beta$ 1 and plasminogen activator inhibitor (PAI)-1 in fibroblasts [57]. Diversion of glyceraldehyde 3-phosphate to a-glycerol phosphate activates protein kinase C (PKC), a pathway with known fibrogenic actions, mediated in part through integrin-dependent fibroblast activation [58].

Increased levels of ROS in diabetic patients cause DNA damage. In patients with type 2 diabetes, oxidative DNA damage was found to correlate with the severity of fibrotic renal tubulointerstitial lesions [59]. The tumor suppressor Homeo-domain interacting protein kinase 2 (HIPK2) is activated in response to oxidative DNA damage and regulates cell survival and fate. Nuclear HIPK2 expression is increased in patients with diabetic nephropathy and may promote EMT, stimulating a fibrotic response [60]. In diabetes, persistent oxidative DNA damage may be due to impaired DNA repair. Hyperglycemia perturbs the ability to repair DNA damage induced by ROS [61], resulting in persistent injury and accelerated aging. Disruption of DNA repair is triggered by a decrease in the NAD<sup>+</sup>/NADH ratio and leads to cellular senescence, tissue inflammation and fibrosis. A recent study documented the role of impaired DNA repair mechanisms in fibrotic remodeling of the lung and kidney, using models of type 1 and type 2 diabetes [62]. Restoration of DNA repair attenuated inflammation and fibrosis and improved organ



**Fig. 2.** Effects of high glucose on fibroblast activity and function. The fibrogenic effects of hyperglycemia may involve several distinct mechanisms, including activation of neurohumoral pathways, induction and activation of growth factors (such as TGF- $\beta$  and PDGFs), stimulation of pro-inflammatory cytokines (such as TNF- $\alpha$  and IL-1 $\beta$ ), generation of AGEs, induction of surface integrins and secretion of matricellular proteins (such as TSP-1, SPARC and CCN2). A central pathway involved in fibroblast activation in response to high glucose involves the generation of reactive oxygen species (ROS). Moreover, high glucose may disrupt DNA repair, thus accentuating the effects of ROS on DNA damage and promoting cellular senescence, inflammatory activation and fibrosis. In response to high glucose fibroblasts proliferate, and undergo activation, secreting collagens, proteases and antiproteases involved in matrix remodeling, and matricellular proteins. Although *in vitro* studies have suggested that high glucose may enhance fibroblast to myofibroblast conversion, to what extent diabetic fibrosis is dependent on myofibroblasts (vs. activated fibroblasts) remains unknown.



function.

#### 4.1.2. Hyperglycemia-mediated neurohumoral activation

The fibrogenic actions of hyperglycemia may involve, at least in part, stimulation of neurohumoral cascades. Hyperglycemia is known to activate the fibrogenic renin-angiotensin-aldosterone system (RAAS) [63] through several distinct mechanisms. First, hyperglycemia stimulates angiotensinogen production [64,65]. Second, high glucose concentrations may increase intracellular angiotensin II expression in fibroblasts [48]. Third, hyperglycemia may upregulate angiotensin II type 1 receptor (AT1R) expression, thus accentuating the fibrogenic Angiotensin II/AT1R pathway [66]. The fibrogenic effects of hyperglycemia-mediated activation of the RAAS may involve both direct actions of angiotensin II and aldosterone on cardiac fibroblasts and indirect effects mediated through induction and activation of TGF- $\beta$  [67].

#### 4.1.3. Hyperglycemia-mediated stimulation of fibrogenic inflammatory cytokines

Hyperglycemia triggers an inflammatory reaction that may play an important role in diabetes-associated fibrosis. High glucose stimulates inflammatory signaling through activation of the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, the molecular platform involved in generation of bioactive Interleukin (IL)-1 $\beta$ . Glucose-mediated activation of the NLRP3 inflammasome is, at least in part, reactive oxygen species (ROS)-dependent [68], and may involve upregulation of the transcription factor E74-like ETS transcription factor 3 (ELF3) [69]. In vivo, inhibition of the inflammasome attenuated diabetes-associated inflammation and fibrosis in the kidney [70] and in the heart [71]. Release of active IL-1 $\beta$  following inflammasome activation may induce other pro-inflammatory cytokines and chemokines (such as TNF- $\alpha$  and CCL2), increasing recruitment and activation of macrophages with fibrogenic properties.

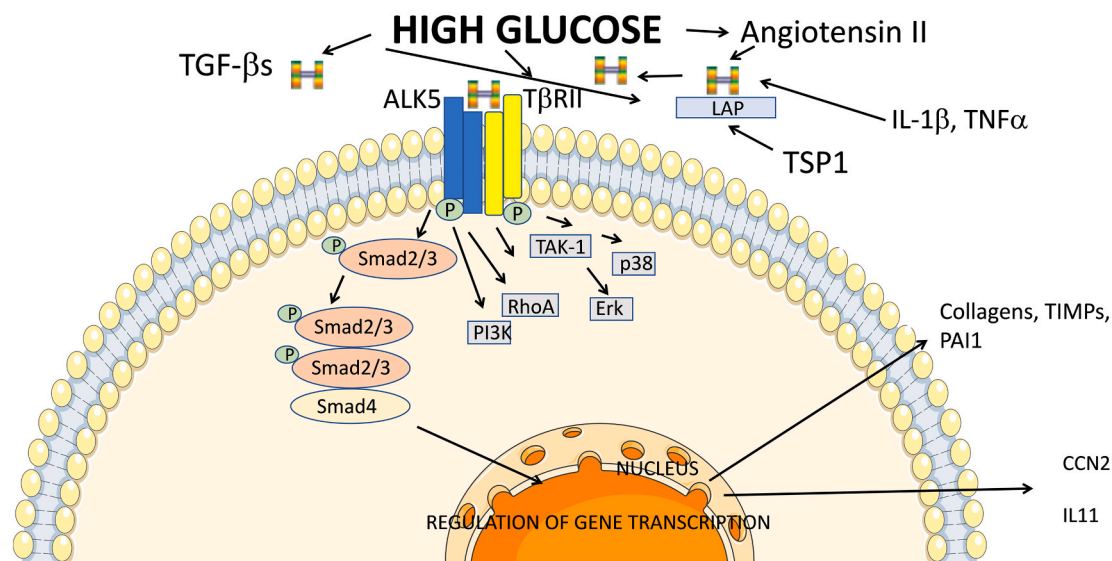
Several lines of evidence suggest that pro-inflammatory cytokines may contribute to the pathogenesis of diabetes-associated fibrosis. First, IL-1 $\beta$  inhibition reduced renal fibrosis in uninephrectomized obese diabetic db/db mice [72]. Second, tumor necrosis factor (TNF)- $\alpha$  antagonism attenuated inflammation and protected from myocardial fibrosis in a rodent model of type 1 diabetes [73]. Third, IL-17 disruption

attenuated renal and myocardial fibrosis in models of streptozotocin-induced diabetes [29,74]. Fourth, IL-6 deletion mitigated myocardial interstitial fibrosis in a model of diabetic cardiomyopathy through downregulation of TGF- $\beta$ 1 [75]. It should be emphasized that pro-inflammatory cytokine signaling has also been implicated in the pathogenesis of metabolic dysfunction in both type 1 and type 2 diabetes [76,77]. Thus, the anti-fibrotic effects of inflammatory cytokine blockade may reflect, at least in part, an improved metabolic profile, rather than direct inhibition of fibrogenic signaling.

#### 4.1.4. Hyperglycemia induces and activates TGF- $\beta$ and transduces fibrogenic Smad-dependent signaling

TGF- $\beta$ s are critically involved in the pathogenesis of tissue fibrosis in a wide range of diseases and experimental models [78]. The 3 TGF- $\beta$  isoforms (TGF- $\beta$ 1, - $\beta$ 2 and - $\beta$ 3) are induced, secreted and activated following injury, and promote myofibroblast conversion, activating a matrix-synthetic program and stimulating secretion of anti-proteases that inhibit matrix degradation (such as Tissue Inhibitor of Metalloproteinase (TIMP)1 and PAI-1) [79,80]. The fibrogenic actions of TGF- $\beta$ s involve activation of a series of intracellular effectors called called Smads, or Smad-independent pathways (Fig. 3). A large body of evidence implicates TGF- $\beta$ s and downstream Smad3-dependent cascades in the pathogenesis of renal and cardiac fibrosis in experimental models of diabetes [81–84].

Although the isoform expression profile and the cellular sources of TGF- $\beta$ s in diabetic tissues remain poorly characterized, several cell types, including fibroblasts, macrophages, epithelial cells, vascular cells, platelets and organ-specific parenchymal cells may be stimulated upon exposure to high levels of glucose to produce and secrete TGF- $\beta$ s. Hyperglycemia-mediated induction and activation of TGF- $\beta$  signaling cascades in diabetic tissues involve several distinct pathways. First, stimulation of the RAAS may trigger TGF- $\beta$  synthesis and promote its activation from latent stores [64]. Second, high glucose triggers oxidative stress, which induces TGF- $\beta$  expression and activation [85]. Third, induction of pro-inflammatory cytokines in response to high levels of glucose may stimulate de novo synthesis of TGF- $\beta$  and activate latent stores through induction of proteases. Fourth, high glucose may generate a TGF- $\beta$ -activating milieu in the pericellular area, by



**Fig. 3.** The link between hyperglycemia and activation of the TGF- $\beta$  cascade. High glucose potentially stimulates fibrogenic TGF- $\beta$ -mediated cascades through several distinct mechanisms involving increased TGF- $\beta$  synthesis, enhanced activation through upregulation of matricellular proteins (such as TSP-1), activation of angiotensin II, induction of pro-inflammatory cytokines (such as IL-1 $\beta$  and TNF- $\alpha$ ) which stimulate TGF- $\beta$  synthesis, and increased surface expression of TGF- $\beta$  receptors. In diabetic tissues, TGF- $\beta$  exerts its fibrogenic effects predominantly through activation of Smad3; the role of Smad-independent pathways remains poorly understood. Although TGF- $\beta$  exerts potent fibrogenic actions through Smad-dependent regulation of gene expression, some of its fibrogenic actions may be mediated through downstream secreted effectors, such as CCN2 and IL-11.

upregulating integrins involved in TGF- $\beta$  activation and by promoting secretion of specialized matrix proteins (such as thrombospondin-1 and ED-A fibronectin) [8,86] implicated in release of the active dimer from latent TGF- $\beta$  stores. Fifth, glucose has been reported to rapidly induce a rapid externalization of TGF- $\beta$  receptors (T $\beta$ Rs) at the cell surface [87] accentuating fibrogenic TGF- $\beta$ /T $\beta$ R1/Smad3 signaling.

#### 4.1.5. Connective tissue growth factor (CTGF/CCN2) and IL-11 as downstream effectors of fibrogenic TGF- $\beta$ signaling

Some of the fibrogenic actions of TGF- $\beta$  have been attributed to downstream synthesis of other secreted pro-fibrotic mediators, such as the matricellular protein CTGF/CCN2, or IL-11 [88]. CCN2 is induced in diabetic tissues by high glucose both through TGF- $\beta$  stimulation and via TGF- $\beta$ -independent pathways [89] and co-operates with other fibrogenic growth factors (such as TGF- $\beta$ 1 and Insulin-like Growth Factor (IGF)-1) [90] to promote fibroblast activation. CCN2 has been reported to play an important role in diabetic renal fibrosis [91], and may act as a matricellular protein that binds to the structural matrix and accentuates growth factor signaling [92]. Recently, IL-11 has been identified as an important downstream effector of TGF- $\beta$ -induced fibrosis; however, its role in diabetes-associated fibrosis remains poorly documented. In experimental models of diet-induced non-alcoholic steatohepatitis, IL-11 inhibition was reported to reduce fibrosis, attenuating liver dysfunction [93].

#### 4.1.6. Fibrogenic effects of the PDGFs

The members of the PDGF family exert potent fibrogenic actions mediated predominantly through the PDGFR $\alpha$ . In vitro studies and associative in vivo data suggest that high glucose may induce expression of PDGFs [94,95], while enhancing activation of PDGFRs [96,97]. Both PDGF-A and PDGF-B are induced in human diabetic kidneys [98]. Studies using co-culture systems suggested that diabetes may induce PDGF secretion by macrophages stimulating fibroblast proliferation [99]. In diabetic mice, activation of PDGFR $\beta$  in the renal cortex was associated with collagen deposition, and in vitro PDGFR $\beta$  was found to mediate effects of high glucose on collagen synthesis by proximal tubular cells [96]. Considering that epithelial cells may not be a major source of collagen in fibrotic tissues in the absence of EMT, the relevance of these findings is unclear.

#### 4.1.7. Hyperglycemia triggers fibrogenic AGE signaling

Glucose reacts non-enzymatically with aminogroups in proteins, lipids and nucleic acids, to produce advanced glycation end-products (AGEs). Chronic hyperglycemia generates AGEs, which promote irreversible cross-linking of the collagenous structural ECM making it less soluble and stiffer [100]. In addition to their effects on matrix stiffness, AGEs can also regulate signaling responses in many cell types, through binding to the Receptor for AGE (RAGE). Activation of the AGE-RAGE axis stimulates several fibrogenic pathways, through direct activation of extracellular signal-regulated kinase (ERK) signaling [101,102], via activation of fibrogenic growth factors, such as TGF- $\beta$  [103] and CCN2 [104] and through stimulation of nuclear factor (NF)- $\kappa$ B-dependent collagen synthesis [105]. In vivo experiments have suggested an important role for AGE-RAGE in diabetes-associated fibrosis. In a rat model of type 1 diabetes, disruption of the AGE-RAGE axis was found to inhibit renal fibrosis and attenuate nephropathy [106].

#### 4.1.8. Fibrogenic effects of hyperglycemia may involve induction of matricellular macromolecules

In addition to its effects on the structural matrix, hyperglycemia also induces synthesis and secretion of matricellular proteins, a family of structurally unrelated matrix macromolecules that do not play a direct role in tissue structure, but bind to ECM proteins and cell surface receptors, transducing or modulating signaling cascades [107]. Thrombospondin (TSP)-1, the best studied matricellular protein in diabetes, enriches the extracellular matrix in diabetic tissues [108–110] and has

been suggested to mediate fibrogenic actions by activating TGF- $\beta$  [111], by stabilizing the ECM through inhibition of Matrix Metalloproteinases (MMP) activity and by promoting vascular rarefaction [108]. Secreted protein, acidic and rich in cysteine (SPARC) is also induced in diabetic tissues and has been implicated in the pathogenesis of diabetes-associated renal fibrosis [112]. The fibrogenic actions of SPARC may involve accentuation of TGF- $\beta$  signaling or effects on ECM assembly [113].

#### 4.2. The fibrogenic effects of insulin resistance

Insulin resistance, the impaired response of the cells to insulin stimulation, is the hallmark of type 2 diabetes. Although the involvement of perturbed insulin signaling in diabetic fibroblast activation has not been systematically studied, the increased oxidative stress typically associated with insulin resistant states may stimulate a fibrogenic program [114]. Moreover, insulin resistance is centrally involved in the pathogenesis of lipotoxic tissue injury, a condition in which accumulation of harmful lipids perturbs organelle function, causing cell injury and contributing to the development of fibrotic lesions [115].

##### 4.2.1. Lipotoxicity in the pathogenesis of diabetes-associated fibrosis

Insulin resistance is associated with marked attenuation of the antilipolytic effects of insulin and results in breakdown of triglycerides and release of free fatty acids (FFAs) from adipose tissue. Circulating FFAs are taken up by the liver, the heart and other organs, where they accumulate as triglycerides. Moreover, perturbations in fatty acid oxidation and direct stimulation of lipogenesis in specific organs (such as the liver and the heart) may further accentuate lipotoxic injury [116,117]. Although triglycerides are the predominant lipids accumulating in tissues, they are not necessarily the most harmful. Diacylglycerols, ceramides, medium chain acyl-carnitines and saturated long-chain fatty acids (such as palmitate) may be associated with a more adverse toxicity profile [117]. In organ parenchymal cells, lipid excess triggers mitochondrial dysfunction and increases endoplasmic reticulum (ER) stress, causing cell injury and contributing to organ dysfunction. Lipotoxic injury in hepatocytes, cardiomyocytes and renal parenchymal cells releases pro-inflammatory and fibrogenic signals that stimulate resident fibroblasts, ultimately leading to organ fibrosis. In human diabetic kidneys, dysregulation of lipid metabolism genes was associated with heavy lipid deposition in podocytes, endothelial cells, tubular epithelial cells and mesangial cells and with increased expression of collagen and fibrogenic growth factors, such as TGF- $\beta$  [118]. Intracellular lipid deposition in tubular epithelial cells activates a fibrogenic program, contributing to the pathogenesis of diabetes-associated fibrosis [119]. In the heart, lipotoxic cardiomyocyte injury has been consistently associated with fibrosis and strategies attenuating lipotoxic injury also attenuated fibrotic cardiac remodeling [120–122]. In the liver, hepatocyte lipotoxicity triggers release of inflammatory and fibrogenic mediators that may promote hepatic stellate cell activation [123]. A recent study demonstrated that BMP-8B, a member of the TGF- $\beta$  superfamily is upregulated in hepatocytes and hepatic stellate cells in patients with NASH and in mouse models of metabolic hepatic fibrosis [124], reflecting a response to lipotoxic injury. BMP-8B activates a fibrogenic program in hepatic stellate cells through both Smad2/3 and Smad1/5 cascades.

It has been suggested that, in addition to these paracrine actions, harmful lipids may also directly promote a fibrogenic response through activating effects on resident tissue fibroblasts. Unfortunately, very little is known regarding the profile and mechanisms of lipid uptake in diabetic fibroblasts. Thus, the evidence supporting a direct role of lipotoxic injury on fibroblast activation is weak and associative, based predominantly on in vitro experiments. In a model of metabolic liver injury, activation of acetyl-CoA carboxylase, the rate limiting step of de novo lipogenesis, was found to mediate a matrix-synthetic hepatic stellate cell phenotype [125]. In cardiac fibroblasts, palmitate contributed to

activation of a pro-inflammatory phenotype, while reducing synthesis of collagen [126]. Thus, any direct effects of lipotoxic injury on fibroblasts are likely dependent on the biochemical profile of lipid accumulation and may not be uniformly fibrogenic.

#### 4.2.2. The fibrogenic effects of hyperlipidemia

Dyslipidemia is often found in both type 1 and type 2 diabetes. Hypertriglyceridemia and low HDL are the most common serum lipid abnormalities observed in diabetic patients [127]. Studies examining associations between diabetes-associated dyslipidemia and organ fibrosis have produced conflicting results. In a population of Mexican NASH patients, hypertriglyceridemia and hypercholesterolemia were independently associated with advanced liver fibrosis [128]. In contrast, other studies failed to establish independent associations between circulating triglyceride and/or cholesterol levels and the severity of hepatic fibrosis [129]. The conflicting findings may reflect sampling at different stages of the disease and the absence of a close relation between serum lipid changes and the severity of organ-specific lipotoxicity.

Animal model studies further support a potential link between dyslipidemia and fibrosis. In a mouse model, genetic hyperlipidemia accentuated the effects of diabetes on extracellular matrix deposition in the kidney [130]. Moreover, lipid lowering strategies were found to reduce fibrosis in experimental models of diabetes. Lipid lowering with 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors or fibrates attenuated fibrosis in mouse models of diabetic nephropathy [131] and cardiomyopathy [132], increasing adenosine monophosphate-activated protein kinase (AMPK) activation [133] and reducing levels of fibrogenic cytokines and growth factors. Whether these anti-fibrotic effects are due to improved circulating lipid profile, or reflect primary anti-fibrotic and anti-inflammatory actions of the lipid-lowering agents remains unclear.

Perturbations in circulating lipoprotein profiles may have a direct impact on fibroblast activity, thus contributing to the increased fibrosis observed in diabetic subjects. In vitro studies suggest that oxidized LDL directly activates fibroblasts, inducing a matrix-synthetic phenotype [134,135]. HDL, on the other hand may inhibit fibrosis, in part through inhibition of EndMT [136]; thus, the reduced HDL levels typically found in diabetic patients may contribute to the accentuated fibrotic changes. Diabetes-associated HDL modifications may also promote fibrosis. Hyperglycemic conditions induce HDL glycation, resulting in attenuation of the anti-inflammatory properties of HDL [137], thus potentially accentuating inflammation-induced fibrogenic responses in susceptible organs.

#### 4.3. Regulation of fibrosis by adipokines

In obesity and diabetes, adipose tissue acts as an endocrine organ, secreting bioactive mediators called adipokines. The prototypical adipokines leptin and adiponectin have been implicated in regulation of tissue fibrosis in diabetic patients. A large body of in vitro evidence supports the notion that leptin promotes fibroblast activation. Leptin binds to the Ob-R receptor and activates fibroblasts by promoting myofibroblast conversion [138], by inducing collagen synthesis [139] and by stimulating MMP activation [140]. Moreover, leptin may promote fibrogenic actions through induction of growth factors, such as TGF- $\beta$  and CCN2, in macrophages [141], or through activation of a fibrogenic program in vascular cells or parenchymal cells. Unfortunately, studies dissecting cell-specific actions of leptin in models of diabetic fibrosis, in order to document effects in fibrotic remodeling, have not been performed.

In contrast to the pro-inflammatory and fibrogenic actions of leptin, adiponectin attenuates insulin resistance and exerts anti-inflammatory, anti-oxidative and anti-fibrotic actions. Adiponectin-mediated suppression of fibrosis may involve direct actions on fibroblast activity, through activation of AMPK and downstream suppression of Smad-dependent

signaling [142], or via activation of peroxisome proliferator-activated receptor (PPAR) $\alpha$  [143]. The in vivo role of endogenous adiponectin in regulating fibrotic responses in patients with diabetes and obesity is poorly documented. Low adiponectin levels in patients with type 2 diabetes were associated with more extensive hepatic fibrosis [144], consistent with the anti-fibrotic actions of the adipokine. Moreover, exogenous activation of adiponectin/AMPK signaling attenuated fibrosis in models of diabetes. In streptozotocin (STZ)-induced diabetic mice, adiponectin gene therapy suppressed mesangial cell proliferation and renal ECM synthesis [145], and in obese diabetic db/db mice, treatment with an adiponectin receptor agonist attenuated cardiac and renal fibrosis [146].

### 5. Epigenetic regulation of diabetic fibrosis

Epigenetic changes encompass post-transcriptional modifications of histone tails [147], chemical nucleosomal DNA processing [148], and the regulatory effects of non-coding (nc) RNAs [74,149]. All these mechanisms may have a profound influence on fibroblast gene transcription in injured tissues [150,151], and can contribute to the pathogenesis of diabetic fibrosis. Unfortunately, evidence documenting mechanistic links between metabolic perturbations and epigenetic modifications that may trigger fibrogenic activation is limited. Histone tail lysine residues can be acetylated by histone acetyltransferases (HATs). In diabetic rats, increased histone acetylation has been associated with fibrotic myocardial changes [152] and in renal mesangial cells high glucose markedly increased histone acetylation accentuating synthesis of fibrogenic growth factors [153]. Moreover, hyperglycemia-mediated Smad2 acetylation by factor acetyltransferase 300 (FATp300) has been implicated in the pathogenesis of TGF- $\beta$ -stimulated cardiac fibrosis [148]. Histone lysine methylation has also been associated with increased ECM production in mesangial cells exposed to high glucose [147]. Acetyl groups are removed from histone tails by a family of enzymes, the histone deacetylases (HDACs) [154]. In experimental models of diabetic nephropathy [155], and cardiomyopathy [156], HDAC inhibition attenuated tissue fibrosis. Whether the anti-fibrotic actions are due to inhibition of fibroblast activation and proliferation, or reflect indirect effects on renal parenchymal cells or cardiomyocytes, respectively, remains unknown.

A rapidly growing body of evidence suggests that ncRNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are implicated in the pathogenesis of diabetes-associated fibrosis. It has been suggested that high glucose may shift the balance of miRNAs in diabetic tissues (such as the heart and kidney), increasing levels of pro-fibrotic miRNAs (including miR-21 [149], miR-155 [157] and miR-200b/c [158]), and downregulating the antifibrotic miRNAs miR15a/b [159], miR133a [160], miR-23b, miR-29, and miR-30 [161–163]. Some miRNAs, such as miR-192 have been suggested to exert both pro- and anti-fibrotic actions in various models of diabetes-associated fibrosis, depending on the experimental setting [164–166]. The molecular links between metabolic perturbations and alterations of miRNA profiles remain unknown.

miRNAs modulate fibrogenic responses by targeting a wide range of fibrogenic cascades, including ion channels, TGF- $\beta$  signaling responses, angiotensin-mediated pathways, Mitogen-activated Protein Kinase (MAPK), RhoA/Rho-associated Protein Kinase (ROCK), and Myocardin-related Transcription Factor (MRTF)/Serum-response Factor (SRF) [167]. Dissection of molecular pathways regulated by miRNAs in diabetic tissue is hampered by the many different cell types modulated by miRNAs and by their wide range of molecular targets.



## 6. Mechanisms and consequences of organ-specific diabetic fibrosis; similarities and differences between distinct tissues

### 6.1. Diabetes-associated cardiac fibrosis

Development of cardiac fibrosis is often documented in patients with advanced diabetes [168]. In many cases, fibrotic cardiomyopathic changes may be due to the increased incidence of coronary atherosclerosis and ischemic heart disease in diabetic subjects. However, in other cases, interstitial and perivascular fibrotic changes may be independent of other comorbid conditions (such as coronary disease and hypertension), likely reflecting primary activation of cardiac fibroblasts in response to hyperglycemia or insulin resistance, or paracrine effects of cardiomyocytes, vascular cells and macrophages. Studies in animal models of type 1 and type 2 diabetes support the notion that metabolic dysregulation is sufficient to induce myocardial fibrosis in the absence of coronary disease or hypertension. The cellular basis and molecular mechanisms responsible for diabetic cardiac fibrosis remain poorly understood. The expansion of the cardiac interstitium and deposition of collagens in diabetic mice may not require myofibroblast conversion, but is associated with activation of a matrix-synthetic program in cardiac fibroblasts [12]. Hyperglycemia directly activates cardiac fibroblasts, by increasing oxidative stress, stimulating neurohumoral cascades, inducing and activating growth factors and matricellular proteins, and leading to accumulation of AGEs that crosslink the interstitial ECM [67,81,169–172]. Insulin resistance may also contribute to fibrosis by increasing ROS generation and by triggering lipotoxic cardiomyocyte injury [173,174]. ROS-dependent microvascular inflammation may also play an important role in fibroblast activation in diabetic patients [175,176]. Diabetes-associated cardiac fibrosis may increase left ventricular stiffness, and may explain the marked increase in the incidence of heart failure with preserved ejection fraction (HFpEF) observed in diabetic patients. Moreover, diabetes-associated fibrosis of the arterial wall may reduce aortic compliance, perturbing pulsatile hemodynamics and ventricular-arterial interactions, and contributing to the pathogenesis of HFpEF [177]. Advanced diabetic fibrosis may also cause heart failure with reduced ejection fraction (HFrEF) by disrupting matrix:cardiomyocyte interactions with a crucial role in survival and contraction of cardiomyocytes, and may also be implicated in the pathogenesis of both atrial and ventricular arrhythmias [178,179].

### 6.2. Diabetes-associated renal fibrosis

Glomerular and tubulointerstitial fibrosis are commonly found in patients with diabetic nephropathy and may contribute to the development of kidney failure in both type 1 and type 2 diabetes. Glomerular lesions range from thickening of the glomerular basement membrane (GBM) to severe diffuse glomerulosclerosis and mesangial expansion. GBM thickening is a characteristic early lesion that reflects accumulation of collagens IV and V, laminins and fibronectin [180,181]. Development of nodular sclerosis (Kimmelstiel-Wilson lesions) is noted at a later stage of the disease, and is associated with lytic mesangial changes, accompanied by endothelial cell detachment. Finally, advanced glomerulosclerosis may develop, characterized by mesangial accumulation of fibrillar type I and type III collagens. In addition to the glomerular fibrotic pathology, patients with diabetic nephropathy often exhibit interstitial fibrosis and tubular atrophy (IFTA) that may also contribute to progression of renal dysfunction [182].

Hyperglycemia is the main stimulus responsible for activation of a fibrogenic program in the diabetic kidney [183]. Deposition of ECM proteins in the glomeruli and in the interstitium reflects expansion of activated myofibroblasts, the main matrix-producing cells in the diabetic kidney [23]. Stimulation of resident renal fibroblasts, conversion of podocytes or tubular cells through epithelial to mesenchymal transition [103,184] and endothelial to mesenchymal transition [185] may

contribute to myofibroblast accumulation in the diabetic kidney. Moreover, all resident renal cell types (including fibroblasts, podocytes, mesangial cells, pericytes, glomerular endothelial cells, and renal tubular epithelial cells) may promote fibrosis by secreting proinflammatory and profibrotic mediators that trigger a matrix-synthetic program in fibroblasts [15,28]. Immune cells, including macrophages, T cells and mast cells, also expand and become activated in the diabetic kidney. Experimental and clinical studies have suggested both profibrotic and protective actions of inflammatory cells in diabetes-associated fibrosis [186,187].

Although *in vivo* experiments documenting a causative role for fibroblast-specific pathways in diabetic renal dysfunction are lacking, several lines of evidence support the notion that fibrogenic activation may be involved in the pathogenesis and progression of diabetic nephropathy. First, in experimental models of diabetes, inhibition of fibrogenic pathways, such as AGE/RAGE [106], chemokine signaling [188], oxidative stress [189] and TGF- $\beta$  [24,83] attenuated renal dysfunction and delayed progression of kidney failure. Second, in patients with diabetes, fibrotic changes are associated with worse renal dysfunction [190], and predict adverse outcome [191]. Third, treatment with pharmacologic agents that inhibit neurohumoral fibrosis (such as ACE inhibitors and AT1 blockers) delay renal fibrosis in diabetes [37], and thus attenuates renal dysfunction. The pathologic links between glomerular fibrosis and renal dysfunction are unclear. GBM thickening contributes to proteinuria and glomerular matrix deposition reduces surface for glomerular filtration [192]. Moreover, tubulointerstitial scarring may lead to decreased reuptake of filtered proteins which augments proteinuria [193]. Proteinuria itself has been suggested to increase NF- $\kappa$ B expression in tubular epithelial cells, inducing EMT and accentuating fibrosis [37]. In addition to its effects on kidney function, diabetes-associated fibrosis may also contribute to the pathogenesis of anemia. In diabetic patients, renal interstitial fibrosis and tubular atrophy were associated with anemia due to lower erythropoietin (EPO) production [194]. Reduced EPO production has been attributed to a decrease in the number of EPO-secreting fibroblasts, as these cells undergo myofibroblast conversion [195].

### 6.3. Hepatic fibrosis

Patients with diabetes, obesity and the metabolic syndrome may develop non-alcoholic fatty liver disease (NAFLD), a condition associated with hepatic steatosis [196,197]. Portal inflammation is typically found in NAFLD patients and is associated with fibrosis and myofibroblast activation [198]. Non-alcoholic steatohepatitis (NASH), the most aggressive form of NAFLD is characterized by rapid progression, hepatocyte injury, inflammation and prominent fibrosis [199–201].

Hepatic fibrosis in NASH is predominantly mediated through lipotoxic hepatocyte injury. Hyperinsulinemia induced by insulin resistance promotes accumulation of cytotoxic lipids, such as free cholesterol and free fatty acids, in hepatocytes, causing mitochondrial oxidative stress and cellular injury. Generation of free radicals and release of DAMPs by injured hepatocytes trigger an innate immune response, activating Kupffer cells, and stimulating leukocyte recruitment [202,203]. Oxidative stress and macrophage-driven inflammation have been suggested to activate hepatic stellate cells (HSCs), promoting their conversion to matrix-synthetic myofibroblasts [204]. In addition to the indirect effects of hepatocyte injury, hyperinsulinemia may directly activate HSCs, promoting a proliferative collagen-producing phenotype [205,206]. Other cell types, including portal fibroblasts, bone marrow-derived fibroblasts, endothelial or epithelial cells have also been suggested to contribute to fibroblast expansion following liver injury [207]; however, their relative contribution in NASH-associated fibrosis remains unclear. Fibrogenic activation of HSCs and deposition of ECM proteins may perturb hepatocyte and endothelial cell function, decreasing transport of substances from the sinusoid to the hepatocytes, and causing hepatic dysfunction [208]. Moreover, HSCs contribute to

hepatic fibrosis by secreting fibrogenic cytokines and growth factors, such as TGF- $\beta$  and CCN2 [209,210]. The significance of hepatic fibrosis in progression of liver dysfunction in diabetic patients is supported by its adverse prognostic implications. In NAFLD patients, fibrosis predicts all-cause mortality, liver-related mortality, liver transplantation, and liver-related morbidity [211]. Hepatic fibrosis in NAFLD patients is reversible upon treatment with anti-diabetic medications (such as metformin or thiazolidinodiones), or following marked weight loss (in patients with severe obesity) [212–214].

#### 6.4. The role of diabetes in pulmonary fibrosis

Although in mouse models, both type 1 and type 2 diabetes are associated with fibrotic lung disease [62,215], the potential role of diabetes-associated fibrosis in pulmonary fibrotic conditions remains underappreciated. Diabetes does not cause overt pulmonary disease; however, several clinical investigations suggest that diabetics may have subclinical alterations in pulmonary function, elasticity and matrix composition [216–218], and may exhibit increased susceptibility to pulmonary fibrosis [219]. It is plausible that the fibroblast-activating effects of hyperglycemia and insulin resistance may accentuate the consequences of idiopathic pulmonary fibrosis, or other chronic lung conditions; however, specific mechanisms responsible for increased susceptibility have not been identified.

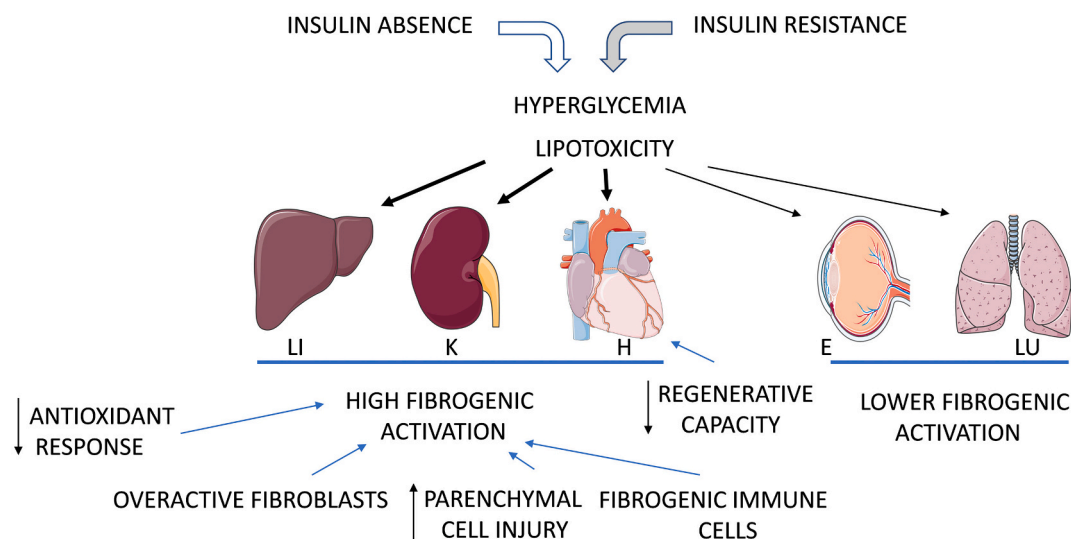
#### 6.5. Fibrosis in diabetic retinopathy

Fibrotic changes are common in advanced diabetic retinopathy and may contribute to the pathogenesis of the disease. During the early non-proliferative phase of diabetic retinopathy, hyperglycemia-induced apoptosis and detachment of pericytes, and endothelial cell death, lead to disruption of the blood-retinal barrier, microvascular leakage, macular edema and microvascular occlusion [220,221]. These microvascular perturbations cause hypoxia and induce inflammation, triggering the proliferative stage of diabetic retinopathy. Glial cells, including Müller cells, the principal glial cell type in the retina, astrocytes and microglia expand and acquire a fibroblast-like phenotype,

producing angiogenic growth factors (such as Vascular Endothelial Growth Factor (VEGF)), and secreting ECM proteins [222–224]. Glial cell-mediated fibrovascular proliferation is characterized by aberrant vessel formation and excessive matrix deposition, and is associated with scar tissue contraction, retinal detachment and visual loss [220]. As the disease progresses, VEGF expression is gradually reduced, while levels of the fibrogenic mediators such as angiotensin II, TGF- $\beta$  and CCN2/CTGF are increased [225–227]. Studies in experimental models of diabetic retinopathy suggest a critical role for fibrogenic growth factors in diabetic retinopathy. Blockade of TGF- $\beta$  and downstream ROCK signaling attenuated cicatricial contraction in experimental models of proliferative vitreoretinal disease [228]. Moreover, in a mouse model of diabetic retinopathy, partial loss of CCN2 attenuated basal lamina thickening in the retinal microvasculature [229].

#### 6.6. What is the basis for the organ specificity of diabetic fibrosis?

Although diabetes-associated activation of major profibrotic pathways has similar characteristics in various tissues, certain organs (such as the kidney, heart and liver) seem to be much more susceptible to fibrotic remodeling in the presence of diabetes (Fig. 4). Several mechanisms may be responsible for the organ-specific patterns of fibrosis in diabetics. First, some organs harbor parenchymal cells more susceptible to lipotoxic or hyperglycemic injury, thus eliciting a potent secondary fibrosis response. Second, different tissues exhibit varying levels of regenerative capacity in response to injury. Tissues with limited regenerative potential, such as the myocardium, rely on fibrosis to repair extensive loss of cells due to injury. Third, fibroblasts the main cellular effectors of fibrosis, and macrophages, major sources of fibrogenic mediators, exhibit marked heterogeneity. Fibrosis-prone tissues may harbor fibroblasts or macrophages with distinct phenotypic characteristics, exhibiting excessive activation in response to hyperglycemia or metabolic dysregulation. Fourth, considering the critical role of oxidative stress in diabetic fibrosis, tissue-specific differences in antioxidant responses may explain distinct responses to similar activating stimuli. Fifth, tissue-specific patterns of fibrogenic growth factor activation may contribute to differences in the timing and intensity of the fibrotic



**Fig. 4.** The basis for the increased susceptibility of the kidney, heart and liver to diabetes-associated fibrosis. Diabetes-associated hyperglycemia, and lipotoxicity exert pro-fibrotic actions on many tissues; however, certain organs such as the liver (LI), kidney (K) and heart (H) seem to exhibit accentuated fibrogenic activation in comparison to other organs, such as the eye (E) and the lung (LU). Several mechanisms may explain the organ-specific fibrotic remodeling in diabetic subjects. First, parenchymal cells in the liver, kidney and heart may be more susceptible to metabolic injury, thus activating secondary inflammatory and fibrogenic reparative responses. Second, organ-specific differences in the cellular composition and growth factor responsiveness of interstitial cells and immune cells may account for higher fibrogenic potential. Third, as oxidative stress is a major driver of fibrotic responses in diabetes, organs with higher endogenous antioxidant mediators may be less susceptible to fibrosis. Finally, organs lacking regenerative capacity (such as the heart) may develop fibrosis in response to the insidious loss of parenchymal cells associated with chronic severe metabolic injury. This figure was created using images from Servier Medical Art (<http://smart.servier.com>).

response. In a model of type 1 diabetes, activation of TGF- $\beta$  signaling occurred earlier in the kidney than in the lung, reflecting higher levels of expression of the inhibitory Smad, Smad7 in pulmonary tissues [230].

## 7. Therapeutic implications: targeting diabetic fibrosis

Considering the broad effects of diabetes-associated fibrosis in several vital organs, including the kidney, heart and liver, strategies attenuating fibrotic remodeling in diabetic patients are attractive and, if effective, may reduce renal, cardiac and hepatic dysfunction. In fact, it is tempting to hypothesize that the beneficial effects of optimal glycemic control, or the protection afforded by agents known to delay progression of renal or cardiovascular disease in diabetic patients (such as ACE inhibitors or statins) may be mediated, at least in part, through attenuation of fibrosis.

### 7.1. The effectiveness of tight glycemic control in preventing organ fibrosis in diabetic patients

Based on the abundant evidence suggesting the critical involvement of hyperglycemia in the pathogenesis of diabetic fibrosis, one could argue that tight glycemic control may be sufficient to abrogate fibrotic remodeling. Several lines of evidence support a close relation between glycemic control and fibrosis-associated complications in diabetic patients. Levels of glycosylated hemoglobin are closely associated with myocardial fibrosis (assessed through cardiac magnetic resonance) in both type 1 and type 2 diabetes patients [231–233]. However, to what extent tight glycemic control can attenuate fibrosis and reduce organ dysfunction remains unclear. Intensive antihyperglycemic treatment delayed progression of diabetic complications (including nephropathy, which is typically associated with fibrosis) in patients with insulin-requiring diabetes [234] and tight glycemic control prevented progression of liver fibrosis in patients with NAFLD [235]. In contrast, in other studies intensive glucose lowering failed to reduce cardiovascular diabetic complications [236,237]. Considering the potential role of glucose-independent mechanisms in the pathogenesis of diabetic fibrosis, additional strategies are likely needed to inhibit and reverse fibrotic remodeling.

### 7.2. Direct anti-fibrotic effects of medications used for glucose lowering

#### 7.2.1. The anti-fibrotic actions of metformin

Metformin is one of the oldest, and most effective antidiabetic medications. In addition to its glucose lowering properties, metformin may inhibit fibrosis through direct anti-fibrotic actions. Metformin acts by stimulating AMP-activated protein kinase (AMPK) and through AMPK-independent mechanisms. Metformin-mediated activation of AMPK may inhibit neurohumoral or growth factor-stimulated fibroblast activation [238], reducing fibrosis in the diabetic liver, heart and kidney [239–241]. In addition, metformin may inhibit fibrosis by interfering with the TGF- $\beta$ /Smad3 signaling cascade [242] and by directly inhibiting formation of AGE-induced collagen cross-links [243]. The significance of the anti-fibrotic properties of metformin in patients remains unclear. Metformin alone or in combination with rosiglitazone did not improve hepatic fibrosis in NAFLD patients [244]. On the other hand, a meta-analysis of heart failure studies suggested that metformin may reduce mortality in HFpEF [245], a condition often associated with fibrosis related to metabolic dysfunction. Whether the protective effects of metformin in this context reflect anti-fibrotic actions has not been investigated.

#### 7.2.2. Incretin-based drugs

Inhibitors of dipeptidyl peptidase-4 (DPP-4) augment circulating levels of incretins such as glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) which in turn increase insulin secretion. Animal model studies suggest that, in addition to their

glucose-lowering actions, DPP-4 inhibitors may also exert direct anti-fibrotic actions. In diabetic renal disease, several DPP-4 inhibitors were shown to attenuate glomerular and tubulointerstitial matrix deposition. For example, linagliptin attenuated glomerulosclerosis and tubulointerstitial fibrosis through inhibition of myofibroblast conversion [246]. Other DPP-4 inhibitors prevented mesangial matrix expansion by anti-inflammatory actions and suppression of TGF- $\beta$  signaling without affecting glucose levels, blood pressure or body weight [247]. The anti-fibrotic effects of gemigliptin and saxagliptin on the kidney were attributed to reduced Smad3 phosphorylation [248], an effect independent of their glucose-lowering effects [249]. Moreover, in a mouse model of NASH due to obesity and insulin resistance, the anti-fibrotic actions of the DPP-4 inhibitor anagliptin were associated with attenuated macrophage-driven inflammation and were independent of effects on body weight, glucose levels, lipid metabolism and hepatic steatosis [250]. Beneficial effects of DPP-4 inhibitors in models of diabetic cardiomyopathy were also attributed to attenuation of fibrosis and improved diastolic function [174]. In line with results from these animal studies, liraglutide, a GLP-1 agonist, prevented progression of fibrosis in NASH patients [251].

#### 7.2.3. Does protection afforded by sodium-glucose co-transporter-2 (SGLT2) inhibitors involve anti-fibrotic effects?

The protective effects of SGLT2 inhibitors in preventing organ damage in patients with diabetes may be due, at least in part, to attenuation of fibrosis. A large body of evidence has documented that SGLT2 inhibitors reduce fibrotic changes in models of obesity, diabetes and metabolic dysfunction. The anti-fibrotic effects have been attributed to attenuation of inflammation, reduced oxidative stress, decreased activation of fibrogenic growth factors and suppressed neurohumoral signaling. In a model of type II diabetes, the SGLT2 inhibitor canagliflozin attenuated tubular fibrosis, reducing renal macrophage infiltration, inhibiting oxidative stress and decreasing hyperglycemia-associated production of angiotensinogen [252]. In other studies using diabetic nephropathy models, empagliflozin prevented renal fibrosis, by suppressing the AGE-RAGE axis [253] and by decreasing ROS-mediated TGF $\beta$ -1 actions [254]. Dapagliflozin was found to delay progression of renal and liver fibrotic remodeling through attenuation of NF- $\kappa$ B-mediated inflammation and NADPH oxidase-induced oxidative stress [255].

In addition to the extensive animal model data discussed above, several clinical investigations support a link between the anti-fibrotic effects of SGLT2 inhibitors and their beneficial actions in patients with diabetic kidney, heart and liver disease [256]. A systems biological approach suggested that canagliflozin-mediated protection from diabetic nephropathy is independent of any effects on glycemic control, but may be associated with reduced inflammation and fibrosis, as suggested by decreased levels of biomarkers, such as TNF- $\alpha$  receptor 1, IL-6, MMP7, and fibronectin levels [257]. Attenuated fibrosis (and improved diastolic function) may be one of the mechanisms responsible for the protective effects of SGLT2 inhibition on mortality and heart failure hospitalization rates in patients with a high cardiovascular risk [258,259]. Moreover, in type 2 diabetes patients with NAFLD, ipragliflozin, as a second-line antidiabetic therapy, normalized ALT levels and improved the fibrosis score [260].

#### 7.2.4. The anti-fibrotic effects of thiazolidinediones

Thiazolidinediones exert potent favorable metabolic effects, lowering levels of blood glucose and lipids, acting as PPAR $\gamma$  agonists. In addition to these actions, thiazolidinedione-mediated PPAR $\gamma$  activation has been suggested to exert anti-fibrotic actions in the heart, kidney and liver. In the diabetic heart, rosiglitazone attenuated diastolic dysfunction, decreasing myocardial fibrosis through downregulation of RAGE and CCN2 [261] and through inhibition of TGF $\beta$ -1 [262]. In patients with NASH pioglitazone improved advanced fibrosis [263], through effects attributed to reduced expression of fibrogenic growth factors



(such as PDGFs) and attenuated inflammation [264]. In the kidney, glitazones may inhibit fibrosis by attenuating high glucose-induced c-Fos-driven TGF $\beta$ -1 activation in mesangial cells [265].

### 7.3. Anti-fibrotic effects of RAAS inhibition in diabetic patients

Some of the protective actions of RAAS inhibition in diabetic patients may involve anti-fibrotic actions. In animal models, ACE inhibitors, AT1 receptor blockers and aldosterone antagonists ameliorated diabetes-induced renal, myocardial and hepatic fibrosis by decreasing expression and activity of pro-fibrotic growth factors (such as TGF- $\beta$ 1 and PDGFs) [63,266], by reducing oxidative stress [267,268], and by attenuating angiotensin II-mediated inflammation [269]. Although experimental work supports the notion that attenuation of fibrosis is a major mechanism of protection upon RAAS inhibition in models of diabetes, the relative contribution of anti-fibrotic actions of RAAS blockers in patients remains unclear. Some clinical studies in obese patients without additional comorbidities and in patients with metabolic syndrome showed that the effects of aldosterone antagonists on left ventricular systolic and diastolic function are associated with reduction in circulating fibrotic markers [270,271].

### 7.4. The anti-fibrotic effects of lipid lowering agents

In addition to their lipid lowering actions, statins (HMG-CoA reductase inhibitors) may also benefit diabetic patients through their direct anti-fibrotic properties. In a model of diabetic cardiomyopathy, atorvastatin reduced inflammation and fibrosis independently of its LDL-cholesterol lowering capacity; these actions were associated with improved ventricular function [272]. The anti-fibrotic effects of statins in diabetic tissues are not limited to the myocardium. Rosuvastatin decreased hepatic steatosis and fibrosis in a model of diet-induced NASH through effects attributed to attenuated inflammation, reduced oxidative stress and decreased expression of fibrogenic mediators, such as TGF- $\beta$ 1 and CCNs [273]. Moreover, in clinical studies, statins delayed fibrosis progression in NAFLD patients [274].

Several other lipid lowering agents have also been suggested to exert anti-fibrotic actions. Ezetimibe, a cholesterol absorption inhibitor, attenuated diabetic cardiac interstitial fibrosis and coronary arterial thickening by reducing macrophage infiltration and NADPH oxidase-mediated oxidative stress [275]. Fibrates have also been reported to inhibit fibrosis. Several studies in animal models of diabetic fibrosis demonstrated that fenofibrate attenuates fibrosis through effects that may involve PPAR $\alpha$  activation. In obese diabetic Zucker rats, fenofibrate reduced renal tubulointerstitial fibrosis, by reducing macrophage infiltration and attenuating expression of proinflammatory cytokines and activation of the TGF- $\beta$ 1/Smad3 axis [276]. Moreover, in mice fed a high-fat diet or given an overload of free fatty acid-bound albumin, fenofibrate acted as a positive regulator of renal lipolysis, inhibiting oxidative stress, and inflammation, and attenuating renal glomerular and tubulointerstitial fibrosis [277].

### 7.5. Inflammatory cytokines as therapeutic targets in diabetes-associated fibrosis

Animal model studies suggest that several pro-inflammatory cytokines, including IL-1, TNF- $\alpha$ , IL-6 and IL-17 contribute to the pathogenesis of diabetes-associated fibrosis [72,75] and may also be involved in the pathogenesis of metabolic dysfunction [76,77]. Considering the availability of effective biologics to neutralize IL-1, TNF- $\alpha$  or IL-6 actions, these cytokines may be attractive therapeutic targets for patients at risk of fibrosis-associated diabetic complications. Despite the theoretical promise of cytokine targeting, clinical evidence supporting this approach is lacking.

In the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial, treatment with the anti-IL-1 $\beta$  monoclonal antibody

canakinumab lowered the incidence of cardiovascular events [278] and reduced the rates for hospitalization for heart failure and heart failure-related mortality in a population of high-risk cardiovascular patients [279]. Whether reduced heart failure events upon IL-1 inhibition reflect attenuated inflammation-driven fibrosis (a prominent feature of diabetes-associated cardiomyopathy) is unknown. In the subgroup of CANTOS patients with chronic kidney disease, canakinumab did not affect indicators of renal dysfunction [280]; however, the absence of an effect does not exclude protective actions upon early administration, or beneficial effects limited to the subset of diabetic patients with active fibrosis. Canakinumab has been reported to reduce HbA1c levels in diabetic patients [281], but had only transient effects on glycemic control in the CANTOS study [282].

### 7.6. The TGF- $\beta$ superfamily as a therapeutic target in diabetic fibrosis

Considering the central role of the TGF- $\beta$  signaling pathway in diabetes-associated tissue fibrosis, inhibition of TGF- $\beta$ /Smad3 signaling may be effective in attenuating organ dysfunction. However, in patients with advanced diabetic nephropathy, treatment with a neutralizing anti-TGF- $\beta$ 1 antibody had no significant effects on renal function, did not affect proteinuria, and did not reduce biomarkers reflecting matrix remodeling [283]. Despite concerns regarding the adverse consequences of TGF- $\beta$  targeting, side effects were comparable between treatment and placebo groups. The basis for the negative results is unclear but may involve ineffective neutralization, effects of other TGF- $\beta$  isoforms, or may reflect the need for early treatment before established fibrosis develops, in order to achieve therapeutic effects.

### 7.7. Antioxidants for diabetes-associated fibrosis

Hyperglycemia and lipotoxic injury mediate their activating effects on fibroblasts, at least in part, through increased oxidative stress. Thus, it is not surprising that in animal models of diabetes, obesity and metabolic dysfunction, administration of antioxidants attenuated fibrosis and delayed the progression of organ dysfunction [284]. Earlier treatment initiation and prolonged administration were suggested to accentuate the protective effects [285]. However, therapeutic translation of these observations in patients has proved challenging. Antioxidant therapies have failed to improve outcomes in high-risk patients, including diabetic subjects [286].

### 7.8. Reducing the fibrogenic effects of AGEs

Considering the important role of AGEs in stimulating fibrosis and in mediating matrix crosslinking in diabetic tissues, reduction of AGE formation, increased AGE degradation, and cleavage of AGE crosslinks have been proposed as promising therapeutic strategies. The prototypical AGE formation inhibitor aminoguanidine (also known as pimagidine) attenuated vascular protein crosslinking and prevented myocardial collagen accumulation, improving diastolic function in diabetes models [100,287]. Moreover, in models of diabetic cardiomyopathy and nephropathy, AGE cross-link breakers restored collagen solubility [288] and improved functional parameters, when treatment was initiated early [289]. Implementation of these strategies in the clinical context is challenging due to the limited specificity of the agents used, and the broad effects of AGEs on many different cell types. However, several clinical studies have produced promising results. In aged humans with arterial hypertension and vascular stiffening, administration of the non-enzymatic cross-link breaker ALT-711 improved total arterial compliance [290]. Moreover, in the ACTION (A Clinical Trial in Overt Nephropathy of Type 1 diabetes) trial, treatment of type 1 diabetic patients with pimagidine did not decrease overt nephropathy, but reduced proteinuria and progression of retinal damage [291].

## 7.9. Challenges of anti-fibrotic approaches in diabetic patients

Although experimental studies suggest an important role for diabetes-associated fibrogenic responses in organ dysfunction and have identified several promising therapeutic targets, implementation of anti-fibrotic strategies in diabetic patients poses several major challenges. First, the relative contribution of fibrotic alterations to organ dysfunction in patients with diabetes is unclear. Fibrosis represents a common reparative response to injury and in certain settings activated fibroblasts may transduce protective signals that attenuate organ dysfunction [292]. Thus, broad and non-selective inhibition of fibrogenic pathways may be detrimental. Second, protection of diabetic patients from maladaptive fibrotic responses may require early initiation of treatment and prolonged therapy, thus exposing patients to potential adverse effects related to the chronic disruption of fibroblast-driven reparative pathways. Third, although attenuation of diabetes-associated fibrogenic responses in several different organs seems an attractive strategy for prevention of a wide range of diabetic complications, various organs have marked differences in cellular composition. Moreover, homeostatic function involves organ-specific signaling networks. Thus, uniformly protective effects of specific anti-fibrotic strategies in all key organs are unlikely, and organ-specific therapeutic interventions may be required to optimize benefit. Fourth, the animal models used to study the cellular responses and molecular signals involved in diabetic complications have major limitations and do not recapitulate the pathophysiologic heterogeneity of human diabetic populations. Although these models are extremely useful to dissect mechanisms of disease, their ability to predict effectiveness of therapeutic approaches is much more limited.

## 8. Conclusions

Fibrosis plays an important role in the pathogenesis of diabetic complications and contributes to the development of renal, hepatic and cardiac dysfunction. However, despite its high clinical significance, diabetes-associated fibrosis remains poorly understood. Our knowledge on the cell biological basis and the mechanistic underpinnings of diabetic fibrotic remodeling is based predominantly on associative data, and on extrapolation of concepts derived from other fibrotic conditions. There is a need for studies, not only to investigate organ-specific mechanisms of diabetic fibrosis, but also to explore the fundamental links between diabetes-associated metabolic perturbations and fibrogenic signaling.

## Disclosures

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## 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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