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PERSPECTIVE ARTICLE

Fibrosis: Sirtuins at the checkpoints of myofibroblast differentiation and profibrotic activity

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Abstract

Fibrotic diseases are still a serious concern for public health, due to their high prevalence, complex etiology and lack of successful treatments. Fibrosis consists of excessive accumulation of extracellular matrix components. As a result, the structure and function of tissues are impaired, thus potentially leading to organ failure and death in several chronic diseases. Myofibroblasts represent the principal cellular mediators of fibrosis, due to their extracellular matrix producing activity, and originate from different types of precursor cells, such as mesenchymal cells, epithelial cells and fibroblasts. Profibrotic activation of myofibroblasts can be triggered by a variety of mechanisms, including the transforming growth factor-β signalling pathway, which is a major factor driving fibrosis. Interestingly, preclinical and clinical studies showed that fibrotic degeneration can stop and even reverse by using specific antifibrotic treatments. Increasing scientific evidence is being accumulated about the role of sirtuins in modulating the molecular pathways responsible for the onset and development of fibrotic diseases. Sirtuins are NAD^+-dependent protein deacetylases that play a crucial role in several molecular pathways within the cells, many of which at the crossroad between health and disease. In this context, we will report the current knowledge supporting the role of sirtuins in the balance between healthy and diseased myofibroblast activity. In particular, we will address the signalling pathways and the molecular targets that trigger the differentiation and profibrotic activation of myofibroblasts and can be modulated by sirtuins.

KEYWORDS
fibroblast-to-transition, myofibroblast, endothelial-to-mesenchymal transition, epithelial-to-mesenchymal transition, fibrosis, myofibroblast, sirtuin

Abbreviations: AEC, alveolar epithelial cell; ANGII, angiotensin II; AS-IV, astragaloside IV; c-ABL, c-Abelson kinase; CMEC, cardiac microvascular endothelial cells; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; EZH2, enhancer of zeste homolog 2; FLIP, flice-inhibitory protein; FMT, fibroblast-to-myofibroblast transition; FOXO, forkhead box O; HDAC, histone deacetylase; HFD, high fat diet; HKL, honokiol; HSC, hepatic stellate cell; IGF, insulin-like growth factor; iHMC, immortalised human mesangial cells; IPF, idiopathic pulmonary fibrosis; IR, ischemia–reperfusion; KO, knockout; LncSIRT1, long non-coding antisense RNAs for sirt1; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NAMPT, nicotinamide phosphoribosyltransferase; NF-κB, nuclear factor kappa b; NOX4, NADPH oxidase 4; NRF2, nuclear factor erythroid 2 like 2; PDGFRβ, platelet derived growth factor receptor beta; PI3K, phosphoinositide 3-kinase; PPARγ, peroxisome proliferator activated receptor γ; PT, proximal tubule cells; ROS, reactive oxygen species; RSV, resveratrol; Sirt, sirtuin; Smad, mothers-against-decapentaplegic-homolog; TGF-β1, transforming growth factor-β1; TIMP1, tissue inhibitor of metalloproteinase 1; TβRI, TGF-β receptor I; TβRII, TGF-β receptor II; UUO, unilateral ureteral obstruction; Wnt, wingless-related integration site; ZDF, zucker diabetic fatty.

1 | INTRODUCTION

Extracellular matrix (ECM) is a tridimensional network of proteins, glycosaminoglycans and glycoconjugates that fills the intercellular space within and between each tissue of multicellular organisms. ECM provides essential biochemical and biomechanical support to the cells. In particular, ECM allows cells to bind together and associate into different tissues and modulates cell morphology, as well as their migration, proliferation and differentiation. ECM is highly dynamic, constantly undergoing remodelling in response to changes in the local biochemical and mechanical microenvironment. This hallmark of ECM guarantees structural and functional stability to tissues and organs.

1.1 | Extracellular matrix homeostasis

Following damage, cells activate quick repair processes aimed at re-establishing the physical integrity of the tissue. During this phase, at the site of injury, precursor cells transdifferentiate into myofibroblasts, which, in turn, produce a large amount of ECM. Different precursor cells can give rise to myofibroblasts, such as fibroblasts, epithelial and endothelial cells, mesenchymal stem cells, pericytes, preadipocytes and adipocytes.[1] Subsequently, the excess of ECM is degraded and new functional tissue is generated.[2,3] Once the wound heals, myofibroblasts must be eliminated for the resolution of the fibrotic process and an effective repair. However, several circumstances, such as chronic inflammation, long-lasting damage and repeated insults may lead to the persistence of active profibrotic myofibroblasts within the tissue. In such a case, apoptosis evasion, or acquisition of a pro-fibrotic senescent phenotype may occur in myofibroblasts.[1] As a result of the accumulation and the persistence of profibrotic myofibroblasts within tissues, homeostatic imbalance between production and degradation of ECM takes place, thus leading to excessive accumulation of ECM.[1,4]

ECM homeostasis is essential for the proper function of tissues and organs and its dysregulation is associated with several pathological conditions, including fibrotic diseases.[3,5–8]

1.2 | Fibrotic diseases

Fibrotic diseases have high prevalence, complex etiology and still no effective therapy in humans.[9] It has been estimated that fibrosis can account for up to 45% of global deaths in western countries, thus representing a major concern to public health.[10,11] In this context, the scientific community devoted a large effort to identify the underlying molecular mechanisms and potential therapeutic targets.[11] Data collected so far suggest that appropriate therapies can effectively stop or even reverse fibrosis.[11]

Myofibroblast differentiation and profibrotic activation are major factors towards the development of fibrosis, and transforming growth factor-β1 (TGF-β1) is one of the principal triggers.[12]

1.3 | Endothelial-to-mesenchymal, epithelial-to-mesenchymal and fibroblast-to-myofibroblast transitions

Endothelial-to-mesenchymal transition (EndMT), as well as epithelial-to-mesenchymal transition (EMT), are highly dynamic processes that occur also during tissue fibrosis. EndMT and EMT consist in the transdifferentiation of polarised endothelial and epithelial cells, respectively, towards the mesenchymal phenotype, which is characterised also by an increased release of ECM components.[13] Indeed, EndMT and EMT have been demonstrated to play a crucial role in the generation of profibrotic myofibroblasts.[2,14–18] Following injury, fibroblasts at the site of injury activate and undergo a phenotype switch to become myofibroblasts, the so-called fibroblast-to-myofibroblast transition (FMT).[19] EndMT, EMT and FMT can be triggered by TGF-β and induce the generation of active profibrotic myofibroblasts.[2,13,19]

1.4 | Epigenetics

Recent studies unveiled the role of epigenetics in the etiology of fibrotic diseases. Indeed, in the context of liver fibrosis, it has been demonstrated that hepatic stellate cells (HSCs) transdifferentiation into myofibroblast is associated with altered histone acetylation pattern.[20] Similar evidence has been collected in cardiac fibrosis. Epigenetics, and in particular histone acetylation, have been shown as critical determinants for the activation of fibroblasts in myocardial fibrosis.[21] In kidney fibrosis, it has been observed an altered epigenetic pattern of chromatin, including histone acetylation status, which is associated with increased fibrogenic gene expression and fibrosis.[22] Also, the TGFβ-dependent activation of lung fibroblasts is mediated by histone deacetylases (HDACs), which prompt profibrotic gene transcription.[23] Additionally, HDACs have been shown to play an important role in the control of collagen gene expression.[2]

1.5 | Sirtuins

Experimental evidence indicates that the survival of myofibroblasts is affected by metabolic and energy-sensing pathways.[1] NAD^+ metabolism is relevant in the onset and progression of fibrosis, as indicated by a recent study on systemic sclerosis. Indeed, Shi and collaborators demonstrated that altered NAD^+ homeostasis (i.e. reduced NAD^+ levels) is associated with multiple organ fibrosis in mice.[24]

Sirtuins belong to a family of proteins with a common structure that has NAD^+-dependent enzymatic activities, such as deacetylase, desuccinylase, demaloynylase, deglutarylase, long-chain deacylase, lipoamidase and ADP-ribosyltransferase. Sirtuin activity has enzyme-regulatory effects but also plays a fundamental role in modulating gene expression, through the deacetylation of histones and transcription factors.[25] In fact, they belong to the Class III of HDACs. Sirtuins, by removing the acetyl groups from histone tails and transcription factors can promote gene transcription.[21]

TABLE 1 Subcellular localization, enzymatic activity, molecular targets and fibrotic effect of sirtuins

Sirtuin	Subcellular localization	Activity	In vivo/in vitro	Organism/cell	Organ/tissue	Effect on fibrosis	Molecular process	References
Sirt1	nucleus, cytoplasm	deacetylase	In vitro/in vivo	Mouse/FB	Skin	Antifibrotic	Inhibition of the TGF-β1 pathway	2
			In vivo	Mouse	Kidney	Antifibrotic	Inhibition of the TGF-β1 pathway; inhibition of Smad4	26
			In vivo/in vitro	Mouse/MC	Kidney	Antifibrotic	Inhibition of the TGF-β1 pathway	27
			In vitro	MC, CM	Kidney, heart	Antifibrotic	Inhibition of Smad3 and activation of PGC1α	2
			In vivo/in vitro	Mouse/FB	Heart	Antifibrotic	Inhibition of Smad2/3	28
			In vivo	Mouse	Endothelium	Putative antifibrotic	Inhibition of the Wnt/β-catenin signalling	29
			In vitro/in vivo	Mouse/HSC	Liver	Antifibrotic	Activation of PPARγ and EZH2	30
			In vivo	Mouse	Lung	Antifibrotic	Activation of NRF2	31
			In vivo/in vitro	Human/FB	Skin	Profibrotic	Activation of TGF-β1 pathway	32
			In vivo/in vitro	Mouse/FB	Lung	Profibrotic	Activation of Ku70	33
			In vitro	MC	Kidney	Profibrotic	Smad7 inhibition	34
			In vivo/in vitro	Mouse/FB	Kidney	Profibrotic	Activation of EGFR and PDGFR pathways	35
Sirt2	nucleus, cytoplasm	deacetylase	In vivo/in vitro	Human-mouse/ TEC-RIF	Kidney	Profibrotic	Activation of the MDM2 pathway	36
			In vitro	RIF	Kidney	Profibrotic	Activation of EGFR and PDGFR pathways	37
			In vitro	HSC	Liver	Profibrotic	Activation of the ERK/c-MYC pathway	38
Sirt3	Mitochondria	Deacetylase	In vivo/in vitro	Mouse/FB	Skin/lung	Antifibrotic	Inhibition of the TGF-β1 pathway; reduction of ROS	39
			In vivo/in vitro	Mouse/FB	Lung	Antifibrotic	Inhibition of the TGF-β1 pathway; reduction of Smad3 expression	40
			In vivo/in vitro	Mouse/FB	Lung	Antifibrotic	Inhibition of the TGF-β1 pathway; reduction of ROS; reduction of mitochondrial DNA damage	12
			In vivo/in vitro	Mouse/AEC	Lung	Antifibrotic	Reduction of ROS	41
			In vivo	Mouse	Kidney	Antifibrotic	Inhibition of the TGF-β/Smad3 signalling	42
			In vitro/in vivo	Mouse/FB	Kidney	Antifibrotic	Inhibition of the NF-kB/TGF-β1/Smad pathway	43
			In vivo	Mouse	Kidney	Antifibrotic	Activation of FOXO3a	44
			In vivo	Mouse	Heart, liver, kidney and lung	Antifibrotic	Inhibition of the TGF-β1 pathway; activation of GSK3β pathway;	45
			In vivo/in vitro	Mouse/FB	Heart	Antifibrotic	Inhibition of the STAT3-NFATc2 pathway	46

TABLE 1 (Continued)

Sirtuin	Subcellular localization	Activity	In vivo/in vitro	Organism/cell	Organ/tissue	Effect on fibrosis	Molecular process	References
Sirt4	Mitochondria	Lipoamidase, ADP-ribosyl transferase, deacetylase	In vivo	Rat	Liver	Antifibrotic	Inhibition of TGF-β1/Smad pathway	47
			In vitro	FB	Lung	Putative antifibrotic	Regulation of mitochondrial metabolism	10,48
			In vivo/in vitro	Mouse/CM	Heart	Profibrotic	Increase in ROS production	49,50
Sirt5	Mitochondria	Demalonylase, desuccinylase, deglutarylase, deacetylase	In vivo	Mouse	Heart	Antifibrotic	Regulation of mitochondrial metabolism	51
			In vitro	FB	Lung	Putative antifibrotic	Regulation of mitochondrial metabolism	10
Sirt6	Nucleus	Deacetylase	In vivo/in vitro	Mouse/CM-FB	Heart	Antifibrotic	Inhibition of c-Jun; histone deacetylation	51
			In vivo/in vitro	Mouse/CMEC	Heart	Antifibrotic	Activation of the Notch1 pathway	52
			In vivo/in vitro	Mouse/HSC	Liver	Antifibrotic	Inhibition of the TGFβ1-Smad3 pathway	53
			In vivo/in vitro	Rat/HBEC	Lung	Antifibrotic	Inhibition of c-Jun; inhibition of the TGFβ1-Smad3 pathway	54
			In vivo/in vitro	Mouse/HKC	Kidney	Antifibrotic	Inhibition of TIMP1	55
Sirt7	Nucleus	Deacylase, deacetylase	In vitro	FB	Lung	Antifibrotic	Down-regulation of Smad3	56
			In vivo	Mouse	Heart	Antifibrotic	Inhibition of p53	57
			In vitro	OSCC	Skin cancer	Putative antifibrotic	Inhibition of Smad4	58
			In vitro	BCC	Breast cancer	Putative antifibrotic	Inhibition of the TGF-β1 pathway; inhibition of Smad4	59
			In vivo/in vitro	Mouse/FB	Heart	Profibrotic	Activation of the TGF-β1 pathway	60
			In vitro	FB	Heart	Profibrotic	Activation of Smad2 and ERK pathway	61

Abbreviations: AEC, alveolar epithelial cell; BCC, breast cancer cell; c-Jun, v-jun sarcoma virus 17 oncogene homolog; CM, cardiomyocyte; CMEC, cardiac microvascular endothelial cell; c-Myc, v-Myc avian myelocytomatosis viral oncogene homolog; EGFR, endothelial growth factor receptor; ERK, extracellular signal-regulated kinase; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FB, fibroblast; FOXO3a, forkhead box O3; GSk3β, glycogen synthase kinase 3 beta; HBEC, human bronchial epithelial cell; HKC, human kidney cell; HSC, hepatic stellate cell; MC, mesangial cell; MDM2, murine double-minute 2; NFATc2, nuclear factor of activated t cells 2; NFκB, nuclear factor kappa b subunit; NRF2, nuclear factor, erythroid 2-like 2; OSCC, oral squamous cell carcinoma; PDGFR, platelet-derived growth factor receptor; PGC1α, PPARγ coactivator 1 alpha; PPARγ, peroxisome proliferator activated receptor gamma; RIF, renal interstitial fibroblast; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TEC, tubular epithelial cell; TGFβ1, transforming growth factor beta 1; TIMP1, tissue inhibitor of metalloproteinases 1; Wnt, wingless-type MMTV integration site family.

In mammalian cells, seven sirtuins (Sirt1-7) with different cellular localization have been identified (Table 1).[62] The ratio between the intracellular concentration of NAD^+ and NADH, two major energy metabolism molecules, determines their activation status: high NAD^+/NADH ratio prompts sirtuin activation, whereas low NAD^+/NADH ratio leads to sirtuin inactivation.[62] Therefore, sirtuins participate in the cellular energy-sensing pathways.[63,64]

These proteins are evolutionary conserved and control key processes within the cells, including cell cycle, autophagy, gene expression, DNA repair, metabolism and stress resistance.[65–67] Sirtuin activity has emerged as a master regulator of the balance between health and disease in mammals and, particularly, in humans, and has been associated with healthy ageing and increased lifespan.[68–70]

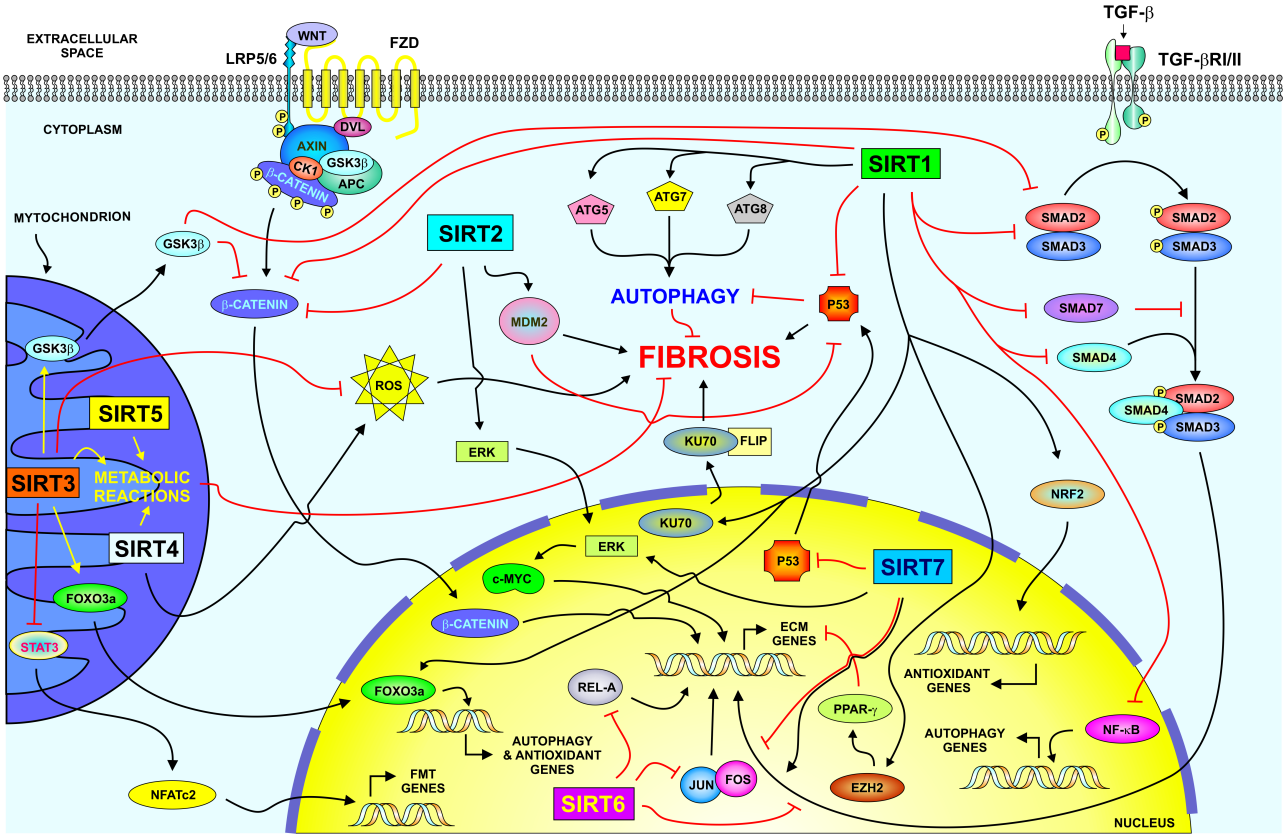

FIGURE 1 Molecular mechanisms underlying the activity of sirtuins on fibrosis. Sirt1 can control the fibrotic processes mainly through the regulation of the TGFβ1 and the Wnt/β-catenin pathways, as well as autophagy, and the ROS balance. Nevertheless, other molecular interactions participate in the complex network of Sirt1-mediated antifibrotic processes. Sirt1 inhibits Smad2, 3 and 4 (key activators of the TGFβ1 pathway), β-catenin and NFkB. Moreover, it activates NRF2, FOXO3a and EZH2, which control the ROS balance, autophagy and the ROS balance, and ECM production, respectively. Sirt1 can also promote fibrosis by activating the TGFβ1 pathway through Smad7 inhibition, and Ku70. Sirt2 induces profibrotic processes by activating the MDM2 and the ERK/c-MYC pathways. Sirt3 blocks fibrosis mainly by inhibiting the Wnt/β-catenin and the TGFβ1 pathways and ROS production. In particular, Sirt3 activates GSK3β, which, in turn, inhibits Smad3 and β-catenin, blocks STAT3-dependent NFATc2 activation, and activates FOXO3a-dependent gene expression. Also, Sirt3 regulates mitochondrial metabolism and stimulates cellular antioxidant defence. Sirt4 activity both promotes and dampens fibrosis by increasing ROS production and regulating mitochondrial metabolism, respectively. The antifibrotic role of Sirt5 has been attributed, so far, to its modulatory effect on mitochondrial metabolic reactions. Within the nucleus, Sirt6 inhibits the expression of profibrotic genes triggered by c-Jun, the Smad complex and Rel-A. Sirt7 exerts its antifibrotic activity by inhibiting p53 and Smad4-dependent gene expression. Conversely, Sirt7 can induce fibrogenic processes by activating the ERK pathway and Smad2-dependent gene expression. APC, adenomatous polyposis coli; AXIN, axis inhibition protein 1; c-Jun, v-jun sarcoma virus 17 oncogene homolog; CK1α, casein kinase 1 alpha 1; c-Myc, v-Myc avian myelocytomatosis viral oncogene homolog; DVL, dishevelled; EMT, endothelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FMT, fibroblast-to-myofibroblast transition; FOXO3a, forkhead box O3 a; FZD, frizzled; GSK3β, glycogen synthase kinase 3 beta; LRP, lipoprotein receptor-related protein; MDM2, murine double-minute 2; NFATc2, nuclear factor of activated t cells 2; NFkB, nuclear factor kappa b subunit; NRF2, nuclear factor, erythroid 2-like 2; PPARγ, peroxisome proliferator activated receptor gamma; ROS, reactive oxygen species; SIRT, sirtuin; SMAD, mothers against decapentaplegic homolog; STAT3, signal transducer and activator of transcription 3; TCF, T cell factor; TGFβ1, transforming growth factor beta 1; WNT, wingless-type MMTV integration site family member

Among all the sirtuins, Sirt1 is the most widely studied. Nevertheless, a deeper understanding of the role of the other six mammalian sirtuins in health and diseases has been progressively accumulated also in fibrotic diseases. Sirtuins modulate several processes involved in fibrosis, such as myofibroblast differentiation and activation, inflammation, senescence and apoptosis.

Here, we review the current knowledge about the intriguing effects of sirtuin activities on critical processes involved in the onset and progression of fibrotic diseases: myofibroblast differentiation and activation. In particular, we report the scientific evidence on the molecular interconnection between sirtuins and profibrotic signalling pathways involved in myofibroblast differentiation and profibrotic activity.

2 | SIRTUINS AND PRO-FIBROTIC SIGNALLING PATHWAYS

Fibrosis can develop in many different tissues.[11] The profibrotic activation of myofibroblasts can be triggered by biochemical stimuli, such as cytokines, peptides and hormones, and mechanical stresses and involves several molecular pathways.[71] The major signalling cascades participating in myofibroblast differentiation and activation are the canonical and non-canonical TGF-β pathway and the wingless-related integration site (Wnt) pathway.[72] Moreover, the mammalian target of rapamycin (mTOR) pathway, the intracellular level of reactive oxygen species (ROS) and autophagy also play an important role in the progression of fibrosis by regulating the EMT, EndMT and the myofibroblast activation.[73–82]

Interestingly, sirtuins can prevent the fibrogenic response through the modulation of many fibrogenic processes, such as FMT, EMT, EndMT and intracellular fibrogenic signallings, such as TGF-β, Wnt and mTOR pathways (Figure 1; Table 1).[10] Moreover, sirtuins regulate other signalling determinants of fibrosis, such as ROS levels, autophagy, insulin-like growth factor (IGF)/protein kinase b alpha (AKT) and nuclear factor kappa b (NF-κB) (Figure 1; Table 1).[10]

2.1 | TGF-β pathway

TGF-β is an important factor in the activation of the pro-fibrotic pathways.[9,83,84] In detail, TGF-β binds to its receptor on the plasma membrane, TGF-β receptor I and II (TβRI and TβRII) and trigger intracellular signalling cascades: the canonical pathway, which relies on the activity of mothers-against-decapentaplegic-homolog (Smad) proteins, and the non-canonical pathway, which does not involve Smad proteins.[9]

Smad proteins are heavily involved in the fibrogenic signalling induced by TGF-β. Indeed, the Smad2/3/4 axis exerts profibrotic effects, whereas the Smad1/5/7 axis is involved in an antifibrotic cellular response.[85,86] Interestingly, Smad3 and Smad7 seem to have opposite activity in the context of renal fibrosis. Smad3 stimulates pro-fibrogenic pathways, whereas Smad7 inhibits them. Besides, Smad3 activation results in Smad7 degradation.[86]

As for the canonical pathway, activated TβRII triggers a phosphorylation cascade that involves TβRI, Smad2 and Smad3. Next, phosphorylated Smad2/Smad3 bind Smad4 in the cytosol, translocate into the nucleus and regulate gene expression.[9] Smad4 mediates the nuclear translocation of the Smad2/3 complex, therefore it promotes the TGF- β1-dependent activation of pro-fibrotic genes.[87,88]

In the non-canonical pathway, following TGF-β stimulation, c-Jun-N-terminal kinase, extracellular signal-regulated kinases (ERK)1/2 and phosphoinositide 3-kinase (PI3K) can be activated[9,89] PI3K activates and elicits the response of p21-activated kinase/c-Abelson kinase (c-ABL) and AKT-mammalian target of rapamycin complex 1 (mTORC1).[9] In turn, c-ABL leads a phosphorylation cascade that includes protein kinase C-δ and friend leukaemia integration 1 transcription factor and ends up with the induction of profibrotic gene expression.[9]

Noteworthy, sirtuins have been demonstrated to interplay with key proteins involved in both the canonical and non-canonical TGF-β signalling pathways.[10,26,90–92] In particular, Sirt1, mainly through its deacetylating activity on Smad proteins, inhibits profibrotic processes.[2,26,31,34] As for Sirt2, it favours the development of fibrosis by activating the ERK pathways.[37,38] Sirt3 dampens the canonical TGF-β signalling pathway, also due to the deacetylation of Smad3, thus reducing the cellular profibrotic responses.[40,42,43,93]

Sirt4 can exert antifibrotic activity by deacetylating Smad4.[47] Interestingly, Sirt6 has interacting partners belonging to both the canonical and non-canonical TGF-β signalling pathways, and its effects are antifibrotic.[51,53,54,94]

Sirt7 regulates Smad2, 3, 4 and ERK proteins, but its activity can be either profibrotic or antifibrotic.[56,58–61]

2.2 | WNT pathway

Another key molecular pathway involved in fibro-proliferative diseases is that driven by Wnt.[95–97] Wnt signalling regulates cell proliferation and differentiation, thus its dysregulation can cause tumours, impaired tissue homeostasis and several developmental diseases.[98–100] Enhanced and sustained activation of the Wnt signal cascade also results in cell differentiation towards a myofibroblast phenotype, thus leading to fibrosis in various mammalian organs.[97,101–103]

Recent studies have reported the modulatory activity of sirtuins on Wnt signalling and its targets.[29,104–106] In detail, Sirt1 binds and deacetylates β-Catenin, thus preventing it to translocate into the nucleus and activate the Wnt-dependent gene expression pattern.[29] Similarly, Sirt2 inhibits Wnt signalling by binding β-Catenin.[104] On the contrary, Sirt3 activity has been associated with an increased Wnt signalling pathway.[105] In the nucleus, Sirt6 forms a molecular complex with β-Catenin and deacetylates the histones in the promoter regions of β-Catenin target genes. As a result, profibrotic gene transcription is abolished.[106]

2.3 | PI3K/AKT and mTOR pathways

The mTOR and PI3K/AKT pathways are involved in the progression of the EMT.[73,74,107] Therefore, suppressing this signalling cascade can

have beneficial consequences on the progression of fibrotic diseases.[108,109]

Both in vitro and in vivo studies demonstrated that the mTORC1 complex is a crucial mediator for the fibrotic response. In dermal fibroblasts from genetically modified mice overexpressing mTORC1, excessive scarring and fibrosis were reported. In particular, proliferation, alpha-smooth muscle actin expression, as well as collagen synthesis, were increased in skin fibroblasts.[110] Moreover, in human lung fibroblasts, the mTORC1/eukaryotic translation initiation factor 4E-binding protein 1 axis mediated the pro-fibrotic effects of TGFβ1.[109] Therefore, the TGFβ1/mTORC1 pathway has been suggested as a potential target for the treatment of fibrotic diseases, such as idiopathic pulmonary fibrosis (IPF).[111]

Interestingly, sirtuins are well-known regulators of the PI3K/AKT and mTOR pathways.[112–116]

Sirtuins regulate the PI3K/AKT signalling pathway through the deacetylation of forkhead box O (FOXO) proteins, which are involved in several important cellular processes, including myofibroblast activation and extracellular matrix production. According to the evidence collected so far, FOXOs may have both activating and inhibitory effects on fibrogenic processes, and Sirt1, as well as other sirtuins, such as Sirt2 and Sirt3, deacetylate FOXOs, thus favouring their nuclear translocation and FOXO-dependent gene expression.[117–122] In particular, in the context of fibrosis, the role of FOXO1 is still controversial, whereas FOXO3a, FOXO4 and FOXO6 have a profibrotic activity.[117–122]

2.4 | ROS signalling

Oxidative stress is a disturbance in the balance between the production and the degradation of intracellular ROS.[123] Many pathological conditions, including fibrotic diseases, are characterised by intracellular accumulation of ROS.[123] The formation of pro-fibrotic myofibroblasts is strongly dependent on intracellular levels of ROS. In fact, sustained intracellular ROS concentration can induce progenitor cells to acquire a fibrogenic phenotype.[124]

Interestingly, Hecker and colleagues demonstrated that aged mice are not able to attenuate and reverse fibrosis. In particular, tissues of aged mice enriched with senescent myofibroblasts with altered redox homeostasis, which was caused by NOX4 (Nox4)-nuclear factor erythroid 2 like 2 (Nrf2) imbalance.[125] Similar observations have been made in the lung of patients affected by IPF where NOX4 was overexpressed and NRF2 down-regulated.[125]

Sirtuins can regulate the redox balance of the cells through the activation of multiple antioxidant pathways and the suppression of those increasing ROS levels and with oxidative activity.[123] Sirtuins control the expression and the activity of pro- and anti-oxidant proteins.[123] Additionally, sirtuins can modulate ROS levels through their deacetylating activity on FOXOs.[126]

Also, the function of sirtuins is dependent on the presence of NAD^+ cofactor, whose intracellular concentration increases upon increased ROS.[124]

Notably, NAD^+ itself is an oxidising coenzyme that participates in the redox reactions of the cells.

2.5 | Autophagy

Autophagy is a fundamental cellular self-degradative process that is needed for energy balance and turnover of macromolecules and organelles.[127–130] It activates in response to reduced nutrient availability and the presence of misfolded proteins or damaged organelles, thus promoting cell function and survival.[127–130] Autophagy is involved in several critical cellular processes, such as senescence, death, antigen activation, genomic stability.[127–130] Therefore, dysregulation of autophagy can lead to several diseases, including fibrosis.[131–133]

It is well known that sirtuins regulate the autophagic machinery in the cells. In particular pieces of evidence have been accumulated on Sirt1, Sirt2, Sirt3 and Sirt6.[112]

Autophagy and sirtuins have many common molecular players such as those involved in the mTOR signalling and FOXO transcription factors.[134,135]

In the context of fibrosis, Hill and collaborators demonstrated that in lung samples from IPF patients, autophagy is repressed. Moreover, in human alveolar epithelial cells inhibition of the autophagic pathway was associated with increased EMT, thus promoting myofibroblast accumulation and activation.[136]

Dysregulation of autophagy can lead to enhanced differentiation and activation of myofibroblasts in lung, liver, kidney and heart tissues, thus being responsible for the development of fibrosis.[75–82]

The association between EMT and autophagy has been also demonstrated for renal fibrosis.[137] In particular, Wang and collaborators showed, both in vitro and in vivo, that activation of the Sirt1–NF-κB pathway induced the activation of autophagy, inhibited EMT in podocytes and attenuated renal fibrosis in a mouse model of diabetes.[137]

Sirt1 protects the kidney from the activation of fibrogenic processes via induction of autophagy.[138–140] At a molecular level, Sirt1 deacetylates several proteins involved both directly and indirectly in autophagy, such as p53, FOXO3a, Atg5, 7 and 8.[112] Conversely, Sirt2 inhibits autophagy by deacetylating FOXO1.[112] Notably, Sirt3 can behave either as an inducer or suppressor of autophagy, depending on the cellular and molecular setting.[112] Also, Sirt6 promotes autophagy. The molecular determinants of its activity are FOXO3 and the Akt–mTOR signalling axis.[112]

The activation of the autophagic machinery can also reduce the accumulation of senescent cells, which can drive fibrogenic processes.[141,142]

Interestingly, it is known that ageing is associated with a decrease in cellular autophagic activity, and it has been accumulated evidence on the age-dependent development of tissue fibrosis.[97,125,136]

Ageing is often associated with an increased number of activated myofibroblasts within tissues and the development of organ fibrosis, which further compromise the health of aged people.[143–148]

Although most of the scientific evidence supports a positive role of autophagy in the control of tissue fibrosis, overactivated autophagy may lead to detrimental effects.[76] Therefore, the autophagic activity must be tightly regulated to exerts beneficial effects, also in the context of fibrosis.

2.6 | SIRT1 and fibrosis

Sirt1, the homolog of yeast SIR2, is a nuclear and cytosolic NAD^+-dependent protein deacetylase that regulates fundamental cellular processes, such as cell cycle, apoptosis, autophagy, ROS generation, cell differentiation and metabolism. Sirt1 can modulate gene expression by deacetylating histones and components of the transcription machinery. Moreover, it controls the activity of several proteins in response to changes in cellular and environmental conditions, including redox balance, nutrient availability and activation of receptor-mediated signalling pathways.[62]

According to experimental evidence collected so far, Sirt1 acts as an antifibrotic protein in many different tissues.

Sirt1 has been found down-regulated in human hypertrophic scar tissue and skin samples from patients affected by systemic sclerosis.[149] Moreover, Sirt1 expression is also decreased in fibrotic kidney and heart, as demonstrated in both human and animal models.[28,150]

The expression of Sirt1 is reduced in myofibroblasts from fibrotic tissue. Moreover, in scar-derived myofibroblasts, the stimulation of Sirt1 inhibited the expression of fibrogenic genes and blocked the profibrotic pathway of TGF-β1.[2]

Studies on mice showed that lack of Sirt1 expression in tubular epithelial cells intensified the fibrotic response following injury.[26] At a molecular level, Sirt1 dampened the pro-fibrotic TGF-β signalling by deacetylating Smad4, thus suppressing the activity of MMP-7.[26]

In a mouse model of wound healing, it has been demonstrated that Sirt1 is essential for effective wound repair, and loss of Sirt1 was associated with abnormal myofibroblast distribution and activation in the skin wound and increased fibrotic reaction in the site of injury.[2,151]

Also, Sirt1 hindered the fibrotic degeneration in patients with chronic obstructive pulmonary disease.[152]

Sirt1 activity can block the transdifferentiation of HSCs towards a myofibroblast phenotype, based on the results obtained in a liver-specific Sirt1 knockout (KO) mouse. At a molecular level, peroxisome proliferator-activated receptor γ (PPARγ) and the enhancer of zeste homolog 2 (EZH2) mediated the antifibrotic activity of Sirt1.[30]

In cultured mesangial cells, as well as in cardiomyocytes isolated from rats, the interaction of Sirt1 with Smad3 and PPAR-γ coactivator-1 inhibited the profibrotic TGF-β1 pathway.[2]

Sirt1 and Wnt pathways are connected. In particular, studies on transgenic mice showed that loss of Sirt1 expression results in enhanced Wnt/β-Catenin signalling.[29]

Sirt1 deficiency is associated with a fibrogenic phenotype in endothelial cells through activation of Wnt and Notch pathways.[153]

Sirt1 is also a well-known suppressor of the mTOR pathway.[113–116]

Vascular fibrosis following angiotensin II (angII) treatment was attenuated by Sirt1 overexpression in mice.[154] In particular, this anti-fibrotic effect of Sirt1 correlated with reduced transcription of TGF-β.[154]

Smad3 mediates the pro-fibrotic signalling of TGF-β. In this context, it has been reported in both in vitro and in vivo that Sirt1 deacetylates and inhibits Smad3.[27] Indeed, Sirt1 up-regulation protected rodents with chronic kidney disease from renal fibrosis via modulation of the TGF-β/Smad3 signalling.[27] Besides, endothelial Sirt1 deficient mice developed early renal fibrosis through down-regulation of the matrix metalloproteinase 14 gene.[155]

Smad7 has been reported to be deacetylated by Sirt1.[34] In SV40-transformed murine mesangial cell, Sirt1 overexpression led to Smad7 ubiquitin-mediated degradation.[34]

In vivo and in vitro studies indicated that Sirt1 deacetylates Smad2/3 and reduces the fibrogenic responses in hearts of pressure overload mice model.[28]

Interestingly, long non-coding antisense RNAs for sirt1 (LncSIRT1) have been identified in fibrotic tissues. LncSIRT1 stabilised Sirt1 mRNA and enhanced Sirt1 expression, and was associated with inhibition of TGF-β1-dependent EMT, thus attenuating pulmonary fibrosis. In a mouse model of bleomycin-induced pulmonary fibrosis LncSIRT1 expression is down-regulated.[156]

Sirt1, by modulating the NRF2/NOX4 axis can reduce the collagen deposition in the lungs of mice that have undergone intestinal ischemia/reperfusion (IR) injury.[31]

Notably, Sirt1 can hinder the effects of advanced glycation end-product (AGE), and, in particular, the induction of EndMT, as demonstrated in human endothelial cells.[157]

In a mouse model of wound healing, it has been demonstrated that Sirt1 blocks the TGFβ1-dependent fibroblast activation and reduces scar tissue formation.[2]

Although most of the pieces of evidence support the idea that Sirt1 expression counteracts the fibrogenic responses within the tissues, opposite results have been also reported.[32,33,35] Indeed, in vitro and in vivo data on systemic sclerosis, showed that Sirt1 down-regulation results in the inhibition of TGF-β signalling, thus leading to antifibrotic effects.[32]

Furthermore, Bulvik and collaborators, through experiments on bleomycin-exposed mice and mouse lung myofibroblasts, showed that Sirt1 increases the levels of flice-inhibitory protein (FLIP) and the Ku70/FLIP complex, by deacetylating Ku70, thus prompting the activation of lung myofibroblasts and the development of fibrosis.[33]

Similarly, in a murine model of renal fibrosis, chemical stimulation of Sirt1 activated renal fibroblast and promoted the development of renal fibrosis. These profibrotic effects of Sirt1 are mediated by the epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor beta (PDGFRβ) signallings.[35]

2.7 | SIRT2 and fibrosis

Sirt2 is a nuclear and cytosolic NAD^+-dependent protein deacetylase, which acts on histones, alpha-tubulin and several other proteins,

including transcription factors. Sirt2 is involved in cell cycle regulation, chromatin remodelling, microtubule dynamics, cell differentiation, metabolism and autophagy.[62]

As for the role of Sirt2 on myofibroblast differentiation and profibrotic activation, scientific evidence is scarce; however, data collected in some studies indicated that Sirt2 exerts mainly a profibrotic activity.

A study on humans and mice demonstrated that Sirt2 expression, through the control of the Double Minute 2 pathway, triggers FMT and fibrosis.[36]

In vitro experiments on HSCs confirmed the profibrotic role of Sirt2 in the liver and suggested Sirt2/ERK/c-MYC axis as responsible for fibrogenic activation.[38]

Other studies on renal fibrogenesis indicated EGFR and PDGFRβ signallings as the ways for Sirt2 to exert its profibrotic activity in renal interstitial fibroblasts.[37]

Also, it has been hypothesised that Sirt2 could exert a pro-fibrotic activity in lung fibrosis.[10]

2.8 | SIRT3 and fibrosis

Sirt3 is a mitochondrial NAD^+-dependent protein deacetylase, which targets many mitochondrial proteins. Sirt3 regulates cellular energy metabolism and the expression of mitochondrial genes.[62]

In the context of fibrosis, and, particularly, of myofibroblast differentiation and profibrotic activation, current research indicates that Sirt3 activity is relevant in antifibrotic processes.

Studies performed on the skin and lung biopsies from patients affected by systemic sclerosis demonstrated that the activity and the expression of Sirt3 are reduced.[39,40] Notably, pharmacological stimulation of Sirt3 reduced the fibrotic reaction both in vitro and in a mouse model of bleomycin-induced fibrosis.[39] In particular, upon Sirt3 activation, TGF-ß signalling, fibrotic gene expression, intracellular ROS generation and myofibroblast differentiation were inhibited.[39]

The expression of Sirt3 is repressed also in lung fibroblasts of humans and mice affected by pulmonary fibrosis. Reduction in Sirt3 levels induced FMT in the lung, as well up-regulation of Smad3 and repression of superoxide dismutase 2 and isocitrate dehydrogenase 2, which are key regulators of intracellular ROS balance. Additionally, Sirt3-deficient mice have an increased risk of developing pulmonary fibrosis. In the same study, enhanced expression of Sirt3 reverted the fibrotic response in the lung.[40]

In a mouse model of cardiac fibrosis, the absence of Sirt3 exacerbated the fibrotic disease.[46] Moreover, the ex-vivo analysis on cells from the same animal model showed an increased propensity of cardiac fibroblasts to transdifferentiate towards a myofibroblast phenotype.[46] The analysis of the molecular signalling involved in this process demonstrated that Sirt3 inhibits the STAT3-NFATc2 pathway.[46]

Experimental data collected from human lung fibroblasts and a bleomycin-induced mouse model of pulmonary fibrosis demonstrated that mitochondrial deacetylase Sirt3 plays a key role in myofibroblast differentiation and the development of pulmonary fibrosis.[12] In vitro, the stimulation with TGF-β1 repressed Sirt3 expression, thus leading to increased levels of ROS and mitochondrial DNA damage.[12] In vivo, similar results have been obtained: Sirt3-KO mice accumulated mitochondrial DNA damage and developed enhanced fibrosis in the lung.[12] Concomitantly, the overexpression of Sirt3 reverted the disease phenotype both in vivo and in vitro.[12]

Jablonski and collaborators reached similar conclusions in a study focused on the role of Sirt3 in IPF.[41] The authors found, in a mouse model of bleomycin-induced lung fibrosis, that loss of Sirt3 results in increased levels of acetylated SOD and mitochondrial 8-oxoguanine DNA glycosylase in the alveolar epithelial cell (AEC). This effect, in turn, inactivated the two enzymes and reduced the ROS defence in the mitochondria of AECs, thus prompting apoptotic cell death and fibrosis.[41]

Studies on diabetic mice showed that loss of Sirt3 favoured the EMT of renal tubular epithelial cells through the TGF-β/Smad3 axis, and exacerbated kidney fibrosis.[42]

Accordingly, Sirt3 expression protects the liver, heart and kidney from the development of fibrosis, as demonstrated in humans and animal models.[158] In particular, studies performed both in vitro and in a mouse model of renal fibrosis demonstrated that up-regulation of Sirt3 activity resulted in a reduced fibrotic response, through regulation of mitochondrial dynamics and the NF-kB/TGF-β1/Smad signalling pathway.[43]

In an angII-induced mouse model of hypertension, Sirt3 hindered the EndoMT and the consequential fibrosis through the deacetylation of FOXO3a.[44]

Besides, Sirt3 activates GSK-3β, which, in turn, dampens the TGFβ1/Smad3 signalling and inhibits myofibroblast activation and fibrotic gene expression.[93] This has been also demonstrated in studies on age-dependent accumulation of fibrotic tissue in mice, where Sirt3 contributes to the reduction of tissue fibrosis with ageing. In particular, TGF-β/glycogen synthase kinase 3β (GSK3β) was inhibited in Sirt3-KO mice, thus leading to hyperactivation of Smad3 and β-Catenin and increased expression of profibrotic genes.[45]

Notably, it has been hypothesised that Sirt3 can repress the EMT also by modulating key enzymes involved in the energy metabolism.[159]

2.9 | SIRT4, SIRT5 and fibrosis

Sirt4 and Sirt5 are mitochondrial NAD^+-dependent enzymes. Sirt4 acts as protein lipoamidase, ADP-ribosyl transferase and deacetylase. Its activity results in the modulation of the function of the target proteins. Sirt4 is involved in the regulation of several different cellular processes, such as cell cycle and metabolism.[62]

Sirt5 is a lysine demalonylase, desuccinylase and deglutarylase. It modulates the activity of the proteins required in different cellular activities, such as metabolic reactions and ROS balance. Sirt5 has a weak deacetylase activity.[62]

Unfortunately, data on the effect of Sirt4 and Sirt5 on fibrotic diseases is scarce. Nevertheless, there are studies indicating Sirt4 as both profibrotic and antifibrotic, and Sirt5 as antifibrotic.

Sirt4 expression is reduced in non-alcoholic fatty liver disease patients, and administration of EX-527, a potent Sirt1 inhibitor, to high-fat diet (HFD) rats was associated with increased levels of Sirt4 and reduced fibrosis. Indeed, the authors suggested that Sirt4/Smad4 might be an important axis for liver fibrosis.[47]

On the contrary, in vitro and in vivo studies on angII-induced cardiac hypertrophy showed that Sirt4 inhibition is associated with reduced cardiomyocyte growth and fibrosis.[49,50] At a molecular level, the Sirt4-dependent increase in ROS production was the major determinant leading to hypertrophic growth, fibrosis and cardiac dysfunction.[49,50]

Sirt4 is also an important negative modulator of glutamine metabolism, which plays an important role in EMT.[48]

Sirt4 and Sirt5 activation has been hypothesised to hinder the fibrotic responses in pulmonary myofibroblasts through their regulatory action on mitochondrial metabolism.[10] Moreover, according to studies performed in animal models, Sirt5 activity protects the heart from age-dependent fibrosis and IR insult.[51]

2.10 | SIRT6 and fibrosis

Sirt6 is a nuclear NAD^+-dependent protein deacetylase, which targets histones and transcription factors. It is important for gene expression, genomic stability and DNA repair and is involved in several metabolic reactions, cellular senescence and apoptosis.[62]

Different studies investigating the role of Sirt6 in myofibroblast differentiation and profibrotic activation indicated that this sirtuin hinders cellular profibrotic reactions.

The level of Sirt6 is reduced in cardiomyocytes of angII, isoproterenol or TAC-treated mice, as well as in samples from patients with heart failure.[51] Moreover, down-regulation or up-regulation of Sirt6 was associated with augmented or attenuated injury-dependent cardiac fibrosis, respectively.[51] At a molecular level, Sirt6-c-Jun interaction and histone acetylation were involved.[51]

Liver fibrosis in patients affected by non-alcoholic steatohepatitis is a critical stage towards the development of serious complications.[160] According to studies on activated HSCs and fibrotic liver, Sirt6 exerts an antifibrotic activity. Its expression is dampened in a fibrogenic milieu.[94] Moreover, Sirt6 deacetylates and inhibits the fibrogenic protein Smad2.[94] In the same cellular system, it has been reported that Sirt6 inhibits the TGFβ1-Smad3 signal transduction pathway, and, as such, blocks the EMT and acts as an antifibrotic agent.[53]

Sirt6 deficient fibroblasts showed up-regulation of TGF-β1, and TGFβRI and an increased tendency to differentiate into myofibroblasts.[161] Sirt6 can inhibit EMT via inactivation TGF-β/Smad signalling in vitro and in vivo.[90–92] It has been reported that this sirtuin hinders the EMT in human bronchial epithelial cells by inhibiting the TGF-β1/Smad3 pathway.[54] Similar results were obtained in a study on endothelium-specific Sirt6-KO mice and cardiac microvascular endothelial cells (CMECs).[52] Zhang and collaborators demonstrated that the absence of Sirt6 exacerbates the fibrogenic phenotype both in vivo and in vitro. In particular, Sirt6 repression favoured the EndMT process probably through the modulation of the Notch1 pathway.[52] Interestingly, the abolition of Sirt6 expression in proximal tubule (PT) cells from streptozotocin-treated diabetic mice, led to an up-regulation of the profibrotic gene tissue inhibitor of metalloproteinase 1 (TIMP1), collagen deposition and tubular basement membrane thickening, thus leading to a worse fibrotic phenotype in the kidney.[55] Similar results have been obtained in another transgenic mouse with PT-specific knockout of the nicotinamide phosphoribosyltransferase (NAMPT) gene, which drives the synthesis of nicotinamide mononucleotide (the NAD+ precursor). Besides, loss of NAMPT resulted in down-regulation of Sirt6.[55] These data demonstrated the importance of the NAD^+ metabolism for the fibrogenic ECM remodelling in diabetic nephropathy.[55]

2.11 | SIRT7 and fibrosis

Sirt7 is a NAD^+-dependent protein-lysine deacylase and deacetylase. It targets histones and non-histone proteins. Sirt7 is localised mainly in the nucleolus of the cells where it acts as a promoter of rDNA transcription. It controls chromatin status, gene expression and DNA damage repair.[62]

The activity of Sirt7 on the onset and development of fibrosis is controversial, due to discordant results reported in different studies.

Sirt7 expression is reduced in human and mouse fibrotic lungs.[56] In addition, Wyman and collaborators demonstrated in vitro that enhanced expression of Sirt7 attenuates FMT. One of the key molecular mediators of the antifibrotic effect Sirt7 is Smad3, whose expression is reduced by Sirt7.[56]

Sirt7 null mice develop cardiac fibrosis, and this effect is mediated by Sirt7-dependent p53 deacetylation.[57]

An inhibitory effect of Sirt7 on EMT and TGF-β signalling has been also demonstrated in oral squamous cell carcinoma and breast cancer cells, where the deacetylation of Smad4, a molecular target of Sirt7, plays a critical role.[58,59]

Conversely, a study on Sirt7-KO mice showed that loss of Sirt7 increases the risk of LV myocardial injury after MI, and was associated with reduced TGFBRI expression and signalling.[60] Moreover, homozygous Sirt7-deficient mice, besides reduced fibrosis showed also compromised potential to contain the damage after myocardial infarction or hind-limb ischemia and ineffective wound healing after skin injury.[60] Sirt7 regulated the signalling of TGF-β through Smad2 and Smad4, and the expression of profibrotic genes.[60]

Moreover, this sirtuin promoted the angII-induced transformation of cardiac fibroblasts into myofibroblasts, in vitro, by activating Smad2 and ERK.[61]

3 | SIRTUINS AS TARGETS OF NATURAL AND SYNTHETIC ANTIFIBROTIC COMPOUNDS

A fine regulation of sirtuin expression and activity has been demonstrated to be beneficial for human health. So far, many different

TABLE 2 Antifibrotic effects of sirtuin modulators through inhibition of myofibroblast differentiation and activation

Compound	In vivo/in vitro	Organism	Organ	Cell	Effect on sirtuins	Molecular mechanism	Effect	References
Resveratrol	In vitro		Heart	FB	Sirt3 activation	Inhibition of TGF-β1-Smad3 signalling	FMT block	162
Resveratrol	In vivo	Rat	Heart/lung/prostate	FB	Sirt1 up-regulation	Inhibition of Wnt signalling	Reduced ECM production	163
Resveratrol	In vivo	Rat	Heart	FB	Sirt1 up-regulation	Inhibition of TGF-β1 signalling	Block of FMT and myofibroblast activation	164
Resveratrol	In vivo	Mouse	Skin	FB	Sirt1 up-regulation	Inhibition of TGF-β1 signalling	FMT block	2
Resveratrol	In vivo	Mouse	Lung	FB	Sirt1 activation	Inhibition of TGF-β1 signalling	FMT block	165
Astragaloside IV	In vitro		Lung	AEC	Sirt1 mRNA stabilisation		EMT block	156
Astragaloside IV	In vitro		Kidney	PD	Sirt1 up-regulation		EMT block	137
Theombrine	In vitro		Kidney	MC	Sirt1 activation	Smad3 deacetylation	Reduced ECM production	166
Theombrine	In vivo	Rat	Kidney		Sirt1 up-regulation	Inhibition of TGF-β1 signalling	Reduced ECM production	166
Honokiol	In vivo	Mouse	Kidney		Sirt3 up-regulation	Inhibition of NFκB/TGF-β1/Smad signalling	Reduced myofibroblast activation and ECM production	43
CUDC-907	In vitro/in vivo	Mouse	Lung	FB	HDAC activity	Inhibition of TGF-β1 signalling	Reduced FMT and ECM production	167
SRT1720	In vivo	Mouse	Kidney		Sirt1 activation	Inhibition of TGF-β1 signalling	Reduced ECM production	168
EX-527	In vivo	Rat	Liver		Sirt4 up-regulation	Inhibition of TGF-β1 signalling	Reduced ECM production	47

Abbreviations: AEC, alveolar epithelial cell; ECM, extracellular matrix; EMT, endothelial-mesenchymal transition; FB, fibroblast; FMT, fibroblast-to-myofibroblast transition; MC, mesangial cell; NFkB, nuclear factor kappa b subunit; PD, podocyte; TGFb1, transforming growth factor beta 1.

natural and synthetic compounds have been demonstrated to be effective in the regulation of sirtuins, also in the context of myofibroblast activation and differentiation (Table 2).[10,114–116,169–173]

One of the most studied natural compounds modulating sirtuin activity is resveratrol (RSV), a polyphenol extracted by grape skin and seeds.[174] This molecule can attenuate several pathological conditions, including fibrosis, and its use has been suggested for preventing various diseases.[2,149,174] In vitro studies showed that RSV can activate Sirt3 and reduce TGF-β1-Smad3 signalling, thus suppressing the differentiation of cardiac fibroblast into profibrotic myofibroblasts.[162] Moreover, this polyphenol can inhibit Wnt signalling, thus reducing the accumulation of fibrotic tissue in the heart, lung and prostate of rats.[163]

In cardiac fibrosis, the role of natural compounds, such as RSV, in the prevention of FMT has been documented.[175] In a rat model of DOXO-induced cardiomyopathy, RSV was effective in attenuating myocardial fibrosis and cardiac fibroblast activation. Furthermore, cardiomyofibroblasts isolated from DOXO-exposed rats and treated with RSV showed increased Sirt1 levels, reduced expression of TGF-β, and decreased phosphorylation of Smad3 compared to cells from RSV-untreated rats.[164]

RSV administration was effective also for the treatment of hypertrophic scar tissues in a mouse model of wound healing.[2] In particular, this polyphenol enhanced the expression of Sirt1 and halted the TGFβ1-induced myofibroblast differentiation and activation in the skin.[2]

Activation of Sirt1 by RSV dampened bleomycin-induced pulmonary fibrosis in mice and prevented the TGF-β-induced differentiation of lung fibroblasts into myofibroblasts.[165]

The treatment with RSV both in vivo and in vitro showed beneficial effects on renal fibrosis by inhibiting EMT through an increase in the expression of Sirt1 and inhibition of the TGF-β pathway.[48]

Astragaloside IV (AS-IV) is a bioactive saponin extracted from the *Astragalus root* that can reduce EMT in pulmonary fibrosis, thus attenuating the disease, by up-regulating LncSirt1 expression.[156]

AS-IV is effective in attenuating renal fibrosis in KK-Ay mice models of diabetes. Additionally, AS-IV in vitro halted glucose-induced EMT in podocytes through activation of autophagy and enhancement of Sirt1 expression.[137]

Theombrine, a purine alkaloid derived from the cacao plant, has been studied for treating diabetic nephropathy. Papadimitriou and colleagues demonstrated that this molecule could rescue the activity of Sirt1 in immortalised human mesangial cells exposed to a high-glucose medium.[166] This effect was associated with reduced acetylation of Smad3 and decreased synthesis of collagen.[166] In streptozotocin-induced diabetic rats, theombrine administration reduced the TGFβ1-driven accumulation of ECM in the kidney through the activation of Sirt1.[166]

Honokiol (HKL), a small polyphenol isolated from the genus Magnolia, can activate Sirt3. Studies in mice undergoing unilateral ureteral obstruction (UUO)-induced renal tubulointerstitial fibrosis demonstrated that HKL reduces myofibroblast activation and ECM deposition. At a molecular level, HKL up-regulated Sirt3 and dampened the NF-κB/TGF-β1/Smad signalling pathway.[43]

In a recent study, CUDC-907, a synthetic compound with both HDAC and PI3K inhibitory activity has been used as antifibrotic and oncosuppressive in lung fibroblasts and myofibroblasts. As result, CUDC-907 exerted beneficial effects against lung fibrosis and cancer.[167]

The use of Sirt1 activators for the treatment of kidney fibrosis has been also demonstrated by Ren and colleagues.[168] Indeed, the authors halted the profibrotic TGF-β1/CTGF signalling pathway in mice with UUO-induced tubulointerstitial fibrosis by administering SRT1720, a Sirt1 activator.[168]

EX-527, a synthetic Sirt1 inhibitor, has been used to treat liver fibrosis in zucker diabetic fatty (ZDF) rats under HFD. This synthetic molecule attenuated fibrosis and reduced the expression of ECM proteins in the liver of HFD-ZDF rats. At a molecular level, EX-527 increased the expression of Sirt4 and reduced the expression of TGF-β, Smad4 and phosphorylated Smad 2/3, all involved in the profibrotic EMT process.[47]

Overall, the evidence collected so far, indicates that sirtuin modulators possess certain fibromodulatory activities.

4 | CONCLUSION

The economical and social burden of fibrotic diseases is rising worldwide, also due to their complex etiology and the limited efficacy of current medical treatments.[11] Therefore, new therapeutical approaches to fibrosis are needed.

In the last decades, sirtuins have emerged as master regulator proteins, that are involved in the control of crucial cellular processes, and whose dysregulation leads to diseases, including fibrosis. Notably, the balance between the beneficial and detrimental effects of their activity relies on several factors, among which the intracellular and extracellular biochemical milieu. This has been demonstrated in the context of cardiac fibrosis, where the activity of Sirt1 can be either healthful or harmful for the heart.[176] Due to the pleiotropic activity of sirtuins, a more detailed understanding of the molecular mechanisms mediating their activity is needed. Furthermore, the studies performed so far showed certain variations in the results, also due to differences in experimental models and protocols.

However, one of the problems encountered when dealing with pharmacological intervention is the off-target effect, and the use of compounds with known pharmacological profiles is clearly of great advantage. The discovery and the development of several natural and synthetic compounds that modulate the activity of sirtuins pawed the way to new areas of intervention, including that for fibrotic diseases. In this context, many sirtuin modulators have been already employed for the treatment of several dysfunctions and diseases, so they might be used in support of anti-fibrotic therapies.[10,70,114–116,169–171,177]

In conclusion, in this review, we present evidence that sirtuins are involved in major determinants of fibrotic diseases, that is myofibroblast differentiation and activation, and that sirtuin modulators might provide an opportunity for the treatment of fibrotic diseases, also in combination with conventional antifibrotic therapies.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

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