Trends in
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Review

The emerging role of metabolism in fibrosis

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The metabolic shift that cancer cells undergo towards aerobic glycolysis was identified as a defining feature in tumours almost 100 years ago; however, it has only recently become apparent that similar metabolic reprogramming is a key feature in other diseases – with fibrosis now entering the fray. In this perspective, an overview of the recent evidence implicating increased glycolysis and glutaminolysis as mediators of fibrosis is presented, with a particular emphasis on the novel therapeutic possibilities this introduces. Furthermore, the impact that metabolic reprogramming has on redox homeostasis is discussed, providing an insight into how this often-overlooked mechanism may drive the pathogenesis.

Highlights

Metabolism has emerged as a major driver of fibrotic diseases.

Glycolytic shifts appear to be a key metabolic switch in stromal cells under fibrotic conditions regardless of aetiology.

Glutaminolysis as well as glycolysis may be a therapeutic target in fibrosis.

Immunometabolites exert antifibrotic effects and may be harnessed for therapeutic gain.

Introduction

Metabolic reprogramming is a well-established event in the development of tumorgenicity; however, evidence is starting to accumulate that it is present in numerous other pathologies, including fibrotic [1–3] and autoimmune [4–6] diseases.

Fibrosis is a pathological feature caused by excessive extracellular matrix (ECM) secretion, resulting in the formation of scar tissue that causes thickening and loss of tissue mobility, culminating in impaired organ function. Chemical or other environmental insults can induce fibrosis; however, it can also be driven by systemic disorders, thought to result from a complex interplay between genetic, epigenetic, and environmental factors. Fibrotic disorders of particular notoriety include systemic sclerosis (SSc), idiopathic pulmonary fibrosis (IPF), liver fibrosis, and nephrogenic systemic fibrosis (Figure 1). A recurring theme in fibrotic events is a prolonged inflammatory response, which alters cellular function and stimulates the fibroblast-to-myofibroblast transition, which underpins many fibrotic disorders [7]. Fibrosis contributes a high level of morbidity and mortality worldwide and has huge economic consequences [8]. In IPF, it has a median survival of 2–5 years from diagnosis. It is estimated that 45% of deaths can be associated with a fibrotic component [9]; thus, fibrosis is a key unmet medical need.

The intracellular mechanisms fuelling the chronic overproduction of ECM are poorly understood, however, which has undermined the search for effective treatments to combat fibrosis. Recently, however, the metabolism has been implicated in multiple profibrotic conditions; hence, this represents an exciting and rapidly emerging area of interest in fibrosis research. In this review, the evidence for metabolic alterations in fibrosis is discussed, with a particular emphasis on the implications this has for the NAD^+/NADH redox balance – a consequence of energy metabolism that is often overlooked but which represents a novel avenue for therapeutic interventions in fibrotic diseases.

Fibrosis: the key cellular players

Fibrosis is characterised by the formation of scar tissue due to increased ECM deposition and can be regarded as aberrant wound healing response that fails to terminate and resolve. Regardless of the aetiology, common principles occur in that there is damage to the tissue followed by unresolved inflammation and then the activation of quiescent cells to myofibroblasts. Myofibroblasts

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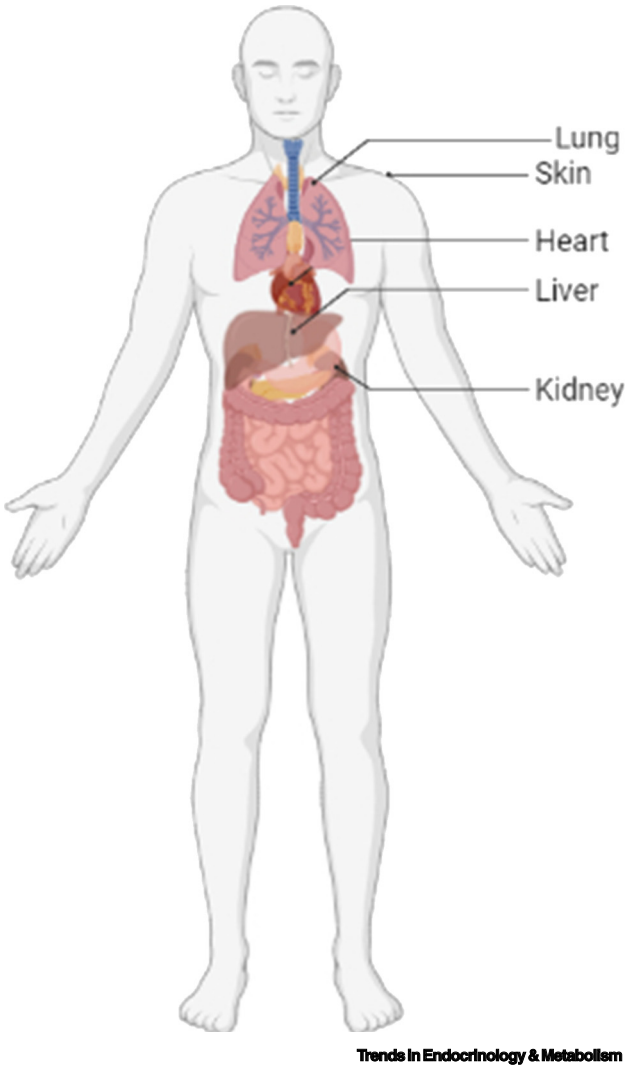

Figure 1. Main organs affected by fibrotic disease. The main organs affected by fibrosis with high prevalence.

are key cells in the pathogenesis of fibrosis and are primarily defined as being contractile due to an increase of alpha smooth muscle actin (α-SMA) and secrete copious amounts of ECM molecules including fibrillar collagen.

Fibrosis is the final common end point of a variety of chronic inflammatory diseases, including the autoimmune diseases such as SSc. Various growth factors and cytokines produced and secreted by innate immune cells such as macrophages and neutrophils can induce fibrosis [10]. As a prime example of a cytokine released from macrophages, interleukin (IL)-1β is sufficient to cause lung fibrosis in mice [11]. In particular, M2-type macrophages are primarily associated with fibrosis. These M2-type macrophages secrete cytokines including IL-4 and IL-13, which then activate quiescent stromal cells to become activated myofibroblasts. Other innate immune cells, such as dendritic cells – sentinels of the immune system – also play a key role in fibrosis development [12], and innate and adaptive immune cells are found often at sites of fibrotic tissue [13], with T regulatory cells being perturbed in fibrosis [14]. Various profibrotic cytokines are

released and activate local fibroblasts or in the lung epithelial-to-mesenchymal transition. IL-17 has also been demonstrated to be profibrotic in multiple organs [15]. One of the most potent cytokines mediating fibrosis is transforming growth factor beta (TGF-β) [16]. TGF-β is secreted in a latent form that is inactive and associated with latent TGF-β binding proteins (LTBPs) associated with the ECM. For TGF-β to cause its effects it has to be activated. One activator is redox stress [17], and thrombospondin-1 through specific repeats activates TGF-β [18].

At the core of the fibrotic response post-stimulation with cytokines is the myofibroblast – the protean cell. This is the chief cell that becomes contractile through actin–myosin bundles and secretes high levels of ECM. In liver fibrosis, this is termed a hepatic stellate cell, and these are critical in liver fibrosis. This normally indolent cell is a key cell type in fibrosis. The deposition of matrix is tightly regulated by enzymes called matrix metalloproteinases (MMPs) and their inhibitors tissue inhibitors of MMPs (TIMPs). The MMPs breakdown the ECM and the TIMPs inhibit MMPs; thus, if the ratio of TIMPs to MMPs is higher, ECM deposition proceeds. TIMP1 and 2 are elevated in hepatic fibrosis [19] and SSc [20]. Collagen crosslinking enzymes like the lysyl oxidase enzymes mediate crosslinking of collagen and are enhanced in fibrotic disease [21]. Matrix stiffness also appears to play a key role in fibrosis; this could work in a feedforward loop in which fibrotic tissue begins to become stiff and this then activates various signalling pathways amplifying fibrosis. This was elegantly demonstrated in IPF, where the diseased matrix amplified fibrosis through enhanced expression of LOX genes that mediated collagen crosslinking, thereby further stiffening the tissue [22]. Mechanical tension can also activate TGF-β from its latent form via integrins, and targeting of integrin αv reduced fibrosis in various animal models [23]. The mechanosensitive proteins Yes-associated protein-1 (YAP1) and TAZ appear to be critical in fibrosis by integrating mechanical cues [24], as does myocardin-related transcription factor-A (MRTF-A) [25]. Another feature of myofibroblasts is their resistance to apoptosis underlying their persistence [26], with resolution of fibrosis associated with enhanced apoptosis [27]. Myofibroblasts appear to show prolonged accumulation and resistance to Fas-mediated death [28]. Furthermore, in many fibrotic conditions epithelial-to-mesenchymal transition occurs, where polarised epithelial cells differentiate to fibroblastic cells (Figure 2). The local microenvironment, specific to that tissue, helps to shape the differentiation of these cells.

Although common pathways may be shared among different target organs (i.e., skin and lung), distinct pathways also exist. Common drivers such as TGF-β appear universal while others are unique to local tissue, and the context must always be borne in mind. SSc is an autoimmune connective tissue disease in which there is widespread fibrosis primarily in the skin, but it can also affect the lungs and kidney [7]; in this condition, again, TGF-β appears to be a central driver of disease.

The Warburg effect: a role in fibrosis?

As already mentioned, upregulated glycolysis, even in the presence of sufficient oxygen, is a defining feature of cancer cells. Although the reason for cancer cells undergoing this metabolic remodelling is controversial, the prevailing theory is that glycolysis increases the production of biosynthetic intermediates that can then be used to support protein synthesis and proliferation [29]. Given that glycolysis yields less ATP than OXPHOS, it would seem peculiar to utilise this pathway, but they cycle this faster and produce synthetic intermediates. Interestingly, the requirement for enhanced protein synthesis and the production of the same biosynthetic intermediates are a hallmark of fibrosis, which requires these intermediates as building blocks for increased ECM production.

The final step in the glycolytic chain yields pyruvate, which then undergoes one of two fates: fermentation to lactate or oxidation to acetyl CoA. The latter enters the tricarboxylic acid (TCA)

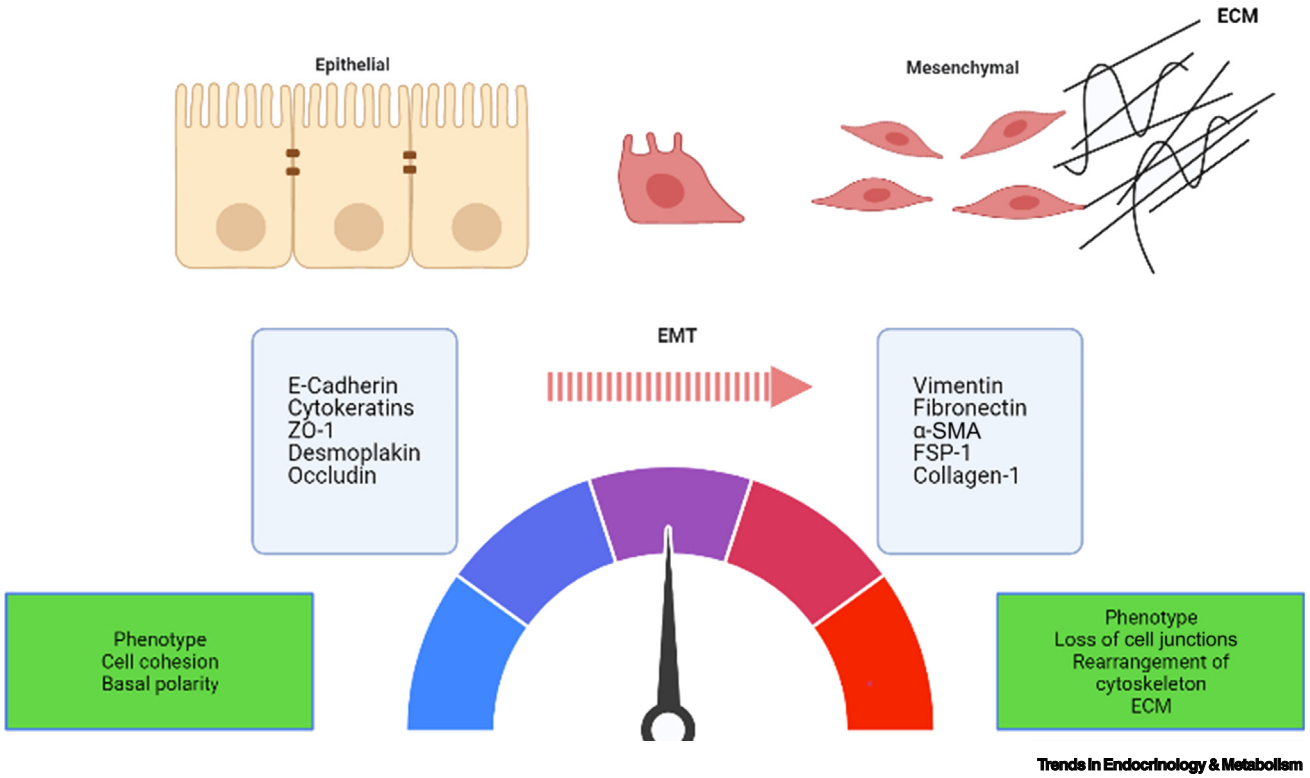

Figure 2. Epithelial–mesenchymal transition. Schematic of epithelial–mesenchymal transition in fibrosis. Epithelial cells lose phenotypical markers (blue boxes) and gain markers of fibroblasts and phenotypes (green boxes). This can be influenced by a variety of factors not shown. Abbreviations: α-SMA, alpha smooth muscle actin; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition.

cycle, which for each acetyl CoA molecule yields a further three NADH and one FAD_2, both of which can subsequently provide electrons to the electron transport chain (ETC) and fuel OXPHOS.

However, an early intermediate in the TCA cycle is α-ketoglutarate, which also an essential precursor for collagen synthesis – a key component of the excessive ECM that defines fibrosis. This therefore identifies a putative mechanism by which increased glycolysis could drive fibrosis, whereby it leads to increased α-ketoglutarate synthesis, which could be redirected from the TCA cycle to the synthesis of collagen (Figure 3).

There is evidence, however, that pyruvate following the alternative route to yield lactate can also promote fibrosis. Kottmann *et al.* found increased lactic acid levels in lung tissue from IPF patients, which also exhibited increased expression of lactate dehydrogenase (LDH) [30]. The same group also found that siRNA-mediated and pharmacological inhibition of LDH prevented the fibroblast-to-myofibroblast transition in TGF-β-treated lung fibroblasts [31]. Particularly notable was their use of gossypol to pharmacologically inhibit LDH; this is a naturally occurring polyphenol that can be derived from cotton and thus produced in abundance. This work has since been augmented by the finding that gossypol protected against radiation-induced pulmonary fibrosis [32] and chemical/diet-induced liver fibrosis in diabetic mice [33], attenuating increased collagen expression and histopathological changes. Concerns regarding the potential genotoxic effects elicited by gossypol have been identified [34]; however, given the interest surrounding LDH inhibition in the context of various cancers, there are numerous other

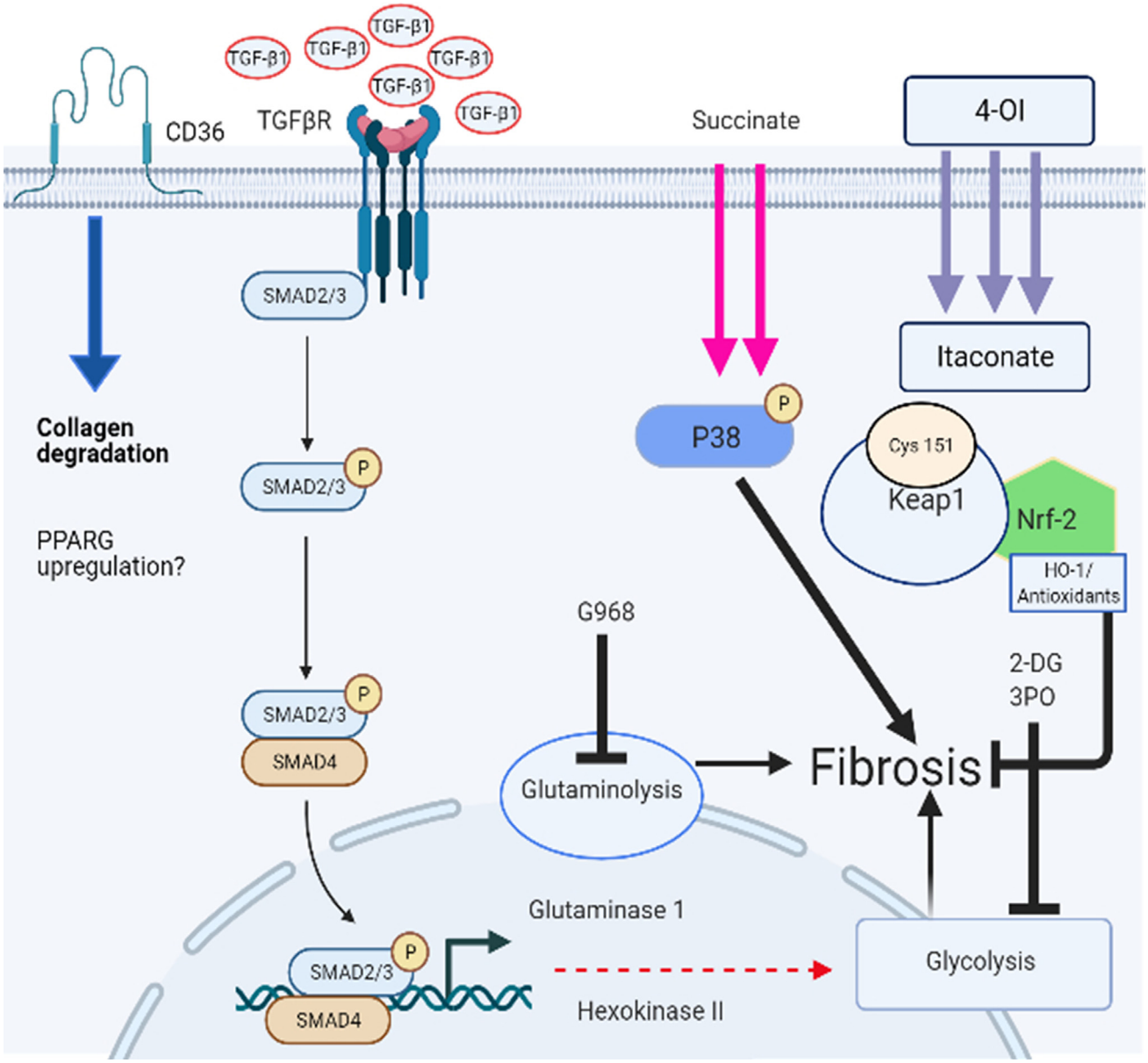

Figure 3. Linking energy metabolism to collagen synthesis. Multiple metabolic pathways can fuel increased collagen synthesis, a process that becomes dysregulated during fibrosis. Both the tricarboxylic acid (TCA) cycle and glutaminolysis can yield α-ketoglutarate, the essential precursor for collagen synthesis. The inhibitor G968 works to block the glutaminase enzymes thus retarding α-ketoglutarate generation. α-Ketoglutarate can also act as a cofactor for demethylase enzymes that site specifically demethylate histone H3 and could possibly alter fibrotic genes in this way. The glycolytic inhibitors 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) and 2-deoxyglucose (2-DG) block fibrosis. Lactate generated from pyruvate can activate transforming growth factor beta (TGF-β) from its latent form covalently linked to LAP. CD36 is a key lipid transport receptor that links energy metabolism to collagen regulation. Reduced surface CD36 diminishes collagen degradation and possibly peroxisome proliferator-activated gamma (PPARγ) downregulation, leading to fibrosis. Succinate generated from the TCA cycle can stimulate its receptor GPR91, leading to activation to a myofibroblast and subsequent fibrosis. Abbreviations: 4-OI, 4-octyl itaconate; HO-1, haemoxygenase-1.

therapeutically viable LDH inhibitors that gossypol could be substituted for [35]. Toxicity of gossypol may limit its clinical usefulness. We have also found elevated lactate in the blister fluid of SSc patients, which was found to upregulate collagen expression, and this could be retarded

by lactate transport inhibitors [36]. Moreover, lactate also modulates T cell effector function, particularly migration [37].

Endothelial-to-mesenchymal transition (similar to epithelial-to-mesenchymal transition) mediation of fibrosis was found to be dependent on glycolysis and mechanistically this led to reduced SIRT3, which ultimately led to increased pyruvate kinase 2 (PKM2) by altering autophagosomal degradation [38].

There is also evidence suggesting that, in addition to lactate production, upregulated glycolysis in a fibrotic environment may also fuel the TCA cycle. TCA cycle metabolites were enhanced in the serum of rats with carbon tetrachloride (CCl_4)-induced liver fibrosis [39], while the key TCA cycle intermediate succinate was also found to be elevated in TGF-β-stimulated lung fibroblasts [40].

TGF-β is the key driver of increased glycolysis in fibrosis

In addition to the growing evidence for the existence of metabolic reprogramming that favours glycolysis, another similarity between fibrotic cells share and tumorigenic cells is the prominent role of TGF-β signalling. TGF-β signalling has been shown to be a key inducer of the shift towards aerobic glycolysis in cancer cells [41,42].

TGF-β is a renowned as a potent inducer of the fibroblast-to-myofibroblast transition and subsequent upregulation of profibrotic genes; hence, its use as the quintessential activator of fibrosis *in vitro*. Although usually regarded as a profibrotic and anti-inflammatory cytokine, TGF-β embodies the term pleiotropic – able to both promote and inhibit processes like inflammation and proliferation depending on the extracellular environment and the cell type it interacts with; in other words, it is context dependant.

In the context of fibroblasts, TGF-β stimulates proliferation, fibroblast-to-myofibroblast differentiation, increased ECM synthesis, and impaired secretion of matrix proteases, combining to create the perfect storm for the development of fibrosis [43]. Under healthy conditions these effects constitute the wound healing response, which is an essential mechanism for tissue repair following damage; however, chronic TGF-β signalling will begin to have a counterproductive outcome. In many ways, this resembles the chronic inflammatory response underlying diseases such as osteoarthritis, multiple sclerosis, and rheumatoid arthritis, whereby a protective mechanism becomes damaging if overactivated.

TGF-β signalling is generally categorised as either Smad-dependent or Smad-independent, emphasising the prominent role that Smad proteins play in transducing TGF-β-driven effects. Of particular importance are Smad2 and 3, which are phosphorylated by TGF-β receptor 1 (TGF-βR1), resulting in the formation of a complex containing Smad2, 3, and 4, which translocates to the nucleus and transcriptionally upregulates profibrotic genes while also indirectly downregulating antifibrotic targets. This allows TGF-β to function as the master controller of fibrosis due to the wide range of genes targeted by the Smad complex, including ECM proteins, profibrotic miRNAs, and TIMPs.

Upregulation of glycolysis is becoming increasingly recognised as a key downstream target of TGF-β in the context of fibrosis. There is evidence for multiple mechanisms driving TGF-β-induced glycolysis in fibroblasts, including increased expression of the glucose transporter GLUT1 [44] and upregulation of hexokinase 2 (HK2) [45]. HK2 is the enzyme responsible for generating glucose 6-phosphate in the first step of glycolysis, and Yin *et al.* demonstrated that HK2 is elevated in IPF fibroblasts and elevates collagen expression via YAP [45]. HIF-1α was

also identified as essential for TGF-β induction of enhanced collagen in human mesangial cells [46], which is noteworthy given that the role HIF-1α plays in facilitating the Warburg effect in cancer is well established [47]. In hepatic fibrosis, in which the hepatic stellate cell is key to the fibrosis, it was demonstrated that glycolysis is critically important and inhibitors that blocked glycolytic reprogramming diminished fibrosis. Interestingly, it appears that hedgehog signalling lies upstream of glycolysis [48]. Keloid fibroblasts also have elevated glycolysis [49]. Furthermore, a recent paper demonstrated enhanced glycolysis in radiation-induced skin fibrosis; this could be blocked by glycolytic inhibitors or the restoration of CD36 – a fatty acid transporter – on fibroblasts [50]. It appears that CD36 regulates the intracellular degradation of collagen. A collagen degradation assay using wild-type or CD36 knockout (KO) fibroblasts identified reduced breakdown of collagen in the CD36 KO cells, indicating its key role in the regulation of turnover [50]. CD36 is brought to the surface by caveolin-1 , which is reduced in SSc [51].

Importantly, a number of glycolytic inhibitors have proved effective in preventing TGF-β induction of fibrotic markers. 2-Deoxyglucose (2-DG) is in many ways considered the classical glycolysis inhibitor, blocking the first step in the glycolytic chain. In TGF-β-treated dermal fibroblasts, 2-DG potently attenuates the increased collagen expression [52] while also displaying impressive efficacy in ameliorating renal fibrosis in the unilateral ureteral obstruction (UUO)-induced mouse model [2]. This was an impressive reduction of fibrosis using a potent HK2 inhibitor *in vivo*. Furthermore, glycolysis inhibition in keloid fibroblasts also reduced ECM [53].

Taking a therapeutic angle, however, complete inhibition of glycolysis would eventually have adverse consequences given its vital role in energy metabolism; hence, an approach that dampens rather than completely stops glycolysis would be more desirable. This can be done using the glycolytic flux inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), which inhibits the cellular capacity to quickly upregulate glycolysis rather than directly inhibiting glycolysis *per se* [54]. In addition to obstructing the TGF-β-induced transition of fibroblasts to profibrotic myofibroblasts *in vitro*, 3PO was able to abrogate the increased collagen and α-SMA expression in the lungs of both bleomycin- and TGF-β-induced pulmonary fibrosis mouse models [40], and specific ablation of PFKFB3 also ameliorated fibrosis. It was also recently demonstrated that PFK3B is elevated in the activation of hepatic stellate cells and that this activation can be blocked with 3PO [55], this was evident in mouse and human cells, and inhibition *in vivo* with two models also ameliorated fibrosis. This is highly suggestive of a possible therapy by tempering rather than demolishing glycolysis.

Glutaminolysis and mTOR

Besides glycolysis, another key metabolic pathway implicated in multiple diseases is glutaminolysis: a two-step process in which glutamine is converted to α-ketoglutarate via glutamate (Figure 3). α-Ketoglutarate is an essential metabolite under growth conditions, functioning as a key precursor for amino acid synthesis and lipid metabolism, while also supporting energy production by feeding into step four of the TCA cycle. Additionally, the transamination of glutamine to glutamate, which constitutes the initial step in glutaminolysis, provides nitrogen required for amino acid synthesis, with glutamate itself also able to be funnelled as a substrate into amino acid synthesis. The increase in succinate following profibrotic stimuli has already been mentioned as a potential indicator of increased TCA cycle activity.

Given that it performs a similar role in facilitating rapid growth, it is perhaps unsurprising that, like glycolysis, glutaminolysis is also regarded as a critical component of cancer development [56]. Similarly, a spike in glutamine metabolism occurs during T cell activation, suggesting it may also be important in autoimmunity [57,58].

One anabolic mechanism thought to be promoted by glutaminolysis but not glycolysis is activation of the mTORC1 complex [59]. mTORC1 is a multisubunit complex that contains as its key catalytic subunit mTOR – a serine/threonine kinase regarded as the 'master regulator' of intracellular anabolic pathways. The relationship between glutaminolysis and mTOR is bidirectional, with α-ketoglutarate activating mTOR via prolyl hydroxylases while the subsequent mTOR-mediated enhancement of nutrient uptake facilitates glutaminolysis through increased glutamine availability. mTOR is also known to enhance glycolysis via HIF-1 [60]; hence, this establishes a potential bridge between glutaminolysis and glycolysis in cancer/fibrosis.

Importantly, mTOR activity can be attenuated by the immunosuppressant rapamycin. The use of rapamycin has proven to be effective in blocking disease pathogenesis in a number of profibrotic rodent models [61–64]. Recently, new inhibitors of mTOR have been found to be antifibrotic in a variety of cell types [65]; rapamycin-insensitive mTORC1 signalling has been found to be a critical node in lung fibrosis.

TGF-β1 has been found to increase glutaminolysis in myofibroblasts and inhibition of glutaminase enzymes reduced ECM [66]. Encouragingly, other inhibitors of glutaminolysis have shown a promising capacity to prevent fibrosis. Three different glutaminase inhibitors been shown to elicit *in vivo* and *in vitro* protection against fibrosis in various organs: CB-839 [67], G968 [52], and BPTES [68–70]. A key question arises: how mechanistically does increased glutaminolysis drive fibrosis? We and others have shown that inhibition of glutaminolysis leads to reduced ECM deposition [52] in fibroblasts; this is also associated with the reduction of α-ketoglutarate. This molecule appears in proline hydroxylation and collagen stabilisation, thus leading to enhanced fibrosis [69]. Another alternative is that another metabolite derived from glutaminolysis contributes to the activation of myofibroblasts. In support of this in cancer cells, glutaminolysis-derived – through noncanonical pathways – aspartate helped to modify the cancer cells by conversion to oxaloacetate [71]. Alternatively, α-ketoglutarate is a cofactor for histone demethylases such as Jumonji C domain-containing demethylase 3 (JMJD3) [72]. These enzymes rely on α-ketoglutarate to site specifically demethylate histones to control gene expression. It could be postulated that in fibrosis the increased glutaminolysis-derived-α-ketoglutarate mediates enhanced demethylation through enhanced activity of JMJD3 at specific regulatory regions thereby promoting fibrosis. Recent studies suggest that JMJD3 activity is important in lung fibrosis [73] and furthermore JMJD3 is elevated in the prototypic fibrotic disease SSc [74], and in diabetic kidney fibrosis JMJD3 and UTX also mediate fibrosis [75]. Furthermore, lysine-specific demethylase 1 (LSD1) which is another α-ketoglutarate-dependent enzyme, is also a specific mediator in pulmonary fibrosis [76]. Also histone acetylation is dependent on citrate lyase converting citrate to acetyl CoA [77], and thus citrate itself through lyase conversion could drive fibrosis. It was shown that siRNA depletion of citrate lyases reduced all markers of fibrosis *in vitro* with or without stimulation with TGF-β1, and a specific inhibitor reduced kidney fibrosis *in vivo* [78]. Many of the animal models used in fibrosis research are initially inflammatory and the fibrotic fibroblasts decrease as the inflammation subsides; it is our view that the myofibroblasts are the chief cell so metabolic intervention could be initiated after the inflammatory phase.

The redox couple NAD^+ and NADH are an important target of metabolic reprogramming

NAD^+ and NADH are essential cofactors for a number of cellular processes. With regard to metabolism, their role in providing reducing equivalents for the ETC is particularly well known. The net gain of NADH from glycolysis and the TCA cycle connects them to OXPHOS, whereby NADH functions as an oxidative cofactor and is converted back to NAD^+. Consequently, the ratio of NAD^+ to NADH infers the equilibrium between OXPHOS and glycolysis: higher NAD^+

suggests a shift towards OXPHOS and vice versa. Importantly, this introduces the relatively novel opportunity to explore NAD^+/NADH-modifying interventions as treatments in disorders involving dysregulated metabolism.

When considering NAD^+/NADH, it is also important to remember that NAD^+ performs a number of vital functions in addition to fuelling ATP production via OXPHOS. As a result, metabolic reprogramming can influence numerous pathways by altering the NAD^+/NADH balance, potentially driving the disease pathogenesis.

A particularly prominent group of enzymes whose activity is tightly controlled by NAD^+ levels is the sirtuin family. The majority of the sirtuins function as NAD^+-dependent deacetylases, which target acetylated residues on histones and other protein substrates, allowing them to modulate a wide array of cellular processes. In the sirtuin family, SIRT1 is believed to play a particularly prominent role in protecting against fibrosis [79–81].

Importantly, NAD^+ is the rate-limiting cofactor for SIRT1; hence, the NAD^+:NADH ratio and thus, by extension, energy metabolism regulate SIRT1 activity. Furthermore, there is evidence that SIRT1 exerts regulatory control of glycolysis [82,83] and OXPHOS [84,85]; hence, NAD^+ levels are likely to represent the fulcrum point in a bidirectional relationship between SIRT1 activity and energy metabolism.

SIRT1 mRNA levels were shown to be decreased in skin biopsies from SSc patients [86], while SIRT1 protein levels were downregulated in chemically induced mouse models of liver fibrosis [87] as well as the UUO model of renal fibrosis [88]. In the latter, the SIRT1 pharmacological activator SRT1720 was effective at reducing fibrosis and attenuating TGF-β signalling [88].

In addition to SRT1720, SIRT1 activity can be enhanced by the polyphenol resveratrol. Resveratrol was shown to potently attenuate the mRNA levels of TGF-β and Smad2/3 in fibroblasts derived from patient scar tissue [89]. Moreover, resveratrol has also displayed its powerful antifibrotic potential *in vivo* by lowering levels of TGF-β and extracellular ECM in UUO rats [90]. TGF-β signalling was also antagonised by resveratrol in bleomycin-treated rats, with increased protein levels of p-Smad2/3 in addition to α-SMA and collagen attenuation [91].

Together this builds a picture of SIRT1 activation eliciting an antifibrotic effect, hence emphasising the importance of maintaining adequate NAD^+ via energy metabolism for SIRT1 function to combat aberrant TGF-β signalling under profibrotic conditions. This also highlights another potential benefit of modifying metabolism in fibrotic disorders: increasing OXPHOS and the NAD^+:NADH ratio, which in turn fuels SIRT1 activity. Additionally, it introduces the exciting prospect of exploring resveratrol as an antifibrotic in disease cohorts, which could be trialled imminently given its already widespread use as a dietary supplement.

Due to its dependence on NAD^+ for its catalytic activity, PARP1 activity is also tightly linked to metabolism and the NAD^+/NADH redox balance. Although the role of PARP1 in cancer is well appreciated given its DNA repair duties, there is accumulating evidence that PARP1 also contributes to fibrotic disorders. PARP1 expression has already been shown to be enhanced in IPF patient fibroblasts [92], while PARP1 inhibition attenuated the expression of proinflammatory and profibrotic markers in bleomycin-treated mice and improved all measured parameters of lung function [93]. Furthermore, Lucarini *et al.* showed that PARP1 inhibition specifically antagonised TGF-β signalling by downregulating circulating TGF-β levels and the expression of p-Smad3 [93].

Pharmacological inhibition of PARP1 also protected against liver fibrosis, in both CCl_4- and bile duct ligation (BDL)-induced mouse models of hepatic fibrosis [94]. Remarkably, not only did PARP1 inhibition protect against the development of fibrosis, but it was also able to reverse the mRNA expression of profibrotic markers and abrogate histopathological changes in the CCl_4-treated mice.

With regard to dermal fibrosis, the PARP1 inhibitor rucaparib has also shown potential, impairing the migration of keloid cells *in vitro* while also reducing their mRNA levels of multiple profibrotic markers [95]. Additionally, rucaparib significantly reduced the size of the keloid tissue in a patient-derived keloid xenograft mouse model [95]. Importantly, with regard to PARP1 inhibition as a potential therapeutic option, three PARP1 inhibitors (olaparib, rucaparib, and niraparib) have already been approved by the FDA for the treatment of certain cancers; hence, these could be repurposed for fibrotic disorders if evidence endorses the antifibrotic outcomes of PARP1 inhibition.

At present, the potential of NAD^+-enhancing treatments to promote healthier ageing is being intensely investigated. To this end, a number of clinical studies have been conducted to determine effective treatments to increase NAD^+. At present, there are two main interventions being focused on for their ability to increase NAD^+: nicotinamide and nicotinamide riboside. Both are precursors for NAD^+ biosynthesis, specifically increasing NAD^+ titres via the NAD^+ salvage pathway and demonstrated to be effective at enhancing NAD^+ titres *in vivo*. Hence, these merit consideration as potential antifibrotic therapeutics, which as dietary supplements with a minimal toxicity profile could be streamlined into clinical trials for fibrotic disorders. There is already a precedent for nicotinamide riboside as an antifibrotic, it having been shown to protect against both CCl_4- and diet-induced liver fibrosis in mice [96,97].

Succinate strikes

Succinate is an intermediate from the TCA cycle, where it is then acted on by succinate dehydrogenase to generate fumarate. However, we now recognise that this can be a signalling molecule, and in macrophages succinate in the cytosol can increase HIF-1α expression and lead to enhanced inflammation through IL-1β induction [98]. Succinate can bind its specific receptor GPR91 (SUNCR1) and cause downstream signalling effects. In dendritic cells, for instance, this enhances immunity [99]. In liver fibrosis, extracellular succinate activates hepatocytes to induce α-SMA and collagen expression and this has also been shown in heart fibrosis [100]. Interestingly, GPR91 expression correlated with the degree of fibrosis in the liver [101] and α-SMA-positive fibroblasts colocalise with GRP91 [102]. Recently, we demonstrated a role for succinate in skin fibrosis by demonstrating that extracellular succinate upregulated collagen robustly [52]. This was not associated, however, with a downstream increase in TGF-β1 levels. Furthermore, collagen 1 mRNA was also reduced by succinate receptor inhibition in hepatic stellate cells [103]. Blockade of GPR91 and succinate signalling maybe a promising therapeutic option in fibrotic disease. This may be true as the signalling for succinate/GPR91 may be only for pathological situations and not normal physiological mechanisms. It is likely that succinate signals through other receptors than those currently known.

Itaconate takes centre stage

Itaconate is a derivative from the TCA cycle that is generated by aconitate decarboxylase 1, also known as immune response gene 1 (IRG1). It was recently shown that, in LPS-activated cells, there is rapid upregulation of Itaconate [104], and knockdown of the gene significantly reduced itaconate levels [105]. It has now emerged that IRG1 and itaconate are important immunoregulatory molecules. It was shown that the electrophilic properties of itaconate lead to upregulation of

the key stress response transcription factor nrf-2 via post-translational modification of Kelch-like ECH-associated protein-1 (Keap1) [106]. Upregulation of nrf-2 leads to an anti-inflammatory response. Subsequently, itaconate or its derivatives appear to be very anti-inflammatory and reduced inflammation in a psoriasis model [107]. Recent studies have identified IRG1 as critical in liver fibrosis, as IRG1 KO mice have an exacerbated liver fibrosis phenotype and administration of an itaconate derivative that is cell permeable, 4-octyl itaconate (4-OI), reduced the exacerbated fibrosis in this mouse model [108] and appears to be dependent on nrf-2. We recently demonstrated that incubation of SSc dermal fibroblasts that have exuberant collagen led to reduced collagen levels in association with upregulation of the nrf-2 target gene haemoxygenase-1 [52], suggestive of an nrf-2-dependent response. This is of interest as nrf-2 is hugely downregulated in fibrotic disease [109]. Recently, Ogger *et al.* defined a role for itaconate in lung fibrosis. They demonstrated that, in IPF, isolated lung macrophages have reduced IRG1 expression, and IRG1 KO mice have exacerbated lung fibrosis in the bleomycin model [110]. Furthermore, treatment of IRG1-deleted mice with itaconate reversed the fibrosis. Our work and the work of others clearly demonstrate a potent antifibrotic effect of itaconate and is suggestive of the use of itaconate derivatives such as 4-OI as antifibrotic compounds (Table 1). It appears that nrf-2 is a critical mediator of the effects, but it is likely that nrf-2-independent effects are at work. It may be speculated that 4-OI inhibits fibrosis through inflammasome inhibition. A recent study demonstrated that 4-OI inhibited the inflammasome by blocking specific protein interactions in macrophages leading to reduced inflammatory cytokine release [111]. It is known in SSc that NLRP3 is elevated, mediating ECM deposition, and it is possible that itaconate could mediated its antifibrotic effect through this suppression. This, of course, requires further investigation, but is a mechanism through which itaconate may mediate its potent behaviours.

Lactylation as a fibrotic regulator

A recently described post-translational modification has been uncovered termed lactylation. Lactate-derived lactylation of specific lysine residues on chromatin epigenetically regulates gene expression in macrophages. The modification of specific lysines with lactate stimulated M2-specific genes in M1 macrophages [112]. This suggests that, in specific lactate-rich environments, macrophages may adopt a more 'wound repair' phenotype after the initial classic 'proinflammatory' M1 polarisation. The reasoning for this is that it may prevent over-excessive inflammation and collateral damage. A recent report from Cui *et al.* demonstrated that conditioned media derived from myofibroblasts stimulated with the profibrotic cytokine TGF-β induced histone lactylation in alveolar macrophages [113]. This specific lactate lysine modification was found in promoters of profibrotic genes such as ARG1. Mechanistically, this was mediated by the acetyltransferase p300, as siRNA knockdown reduced lysine lactylation and reduced the M2-like phenotype [113]. This indicates that myofibroblasts polarise macrophages through epigenetic modifications via increased release of lactate and lactylation, leading to a reparative phenotype that if unresolved would be deleterious. Targeting myofibroblast lactate could be a

Table 1. Role of IRG1 and itaconate in fibrosis models

Organ	Detail[a]	Refs
Liver	IRG1 KO mice had significantly exacerbated fibrosis	[108]
Lung	IRG1 KO mice have exacerbated fibrosis that was reduced with adoptive transfer of IRG1-wild-type macrophages; fibroblasts incubated with itaconate showed reduced ECM and proliferation	[110]
Kidney	Kidney fibrosis UUO model was attenuated with 4-OI treatment; appears to modulate autophagy and ROS generation	[116]
Skin	Incubation of dermal SSc fibroblasts reduced collagen expression and upregulates HO-1[a]	[52]

[a]Abbreviation: HO-1, haemoxygenase-1.

possible therapeutic in fibrotic diseases. However, it is likely that such post-translational modifications to histone proteins have critical homeostatic effects directing normal physiological events; thus, targeting these may have deleterious side effects. With lactylation being such a recently described phenomenon, descriptions of its role are few, but it could represent a novel therapeutic target.

Concluding remarks

Finally, the application of metabolism-targeting therapeutics in fibrotic disease patients needs to be considered (see Outstanding questions). A number of glycolytic inhibitors are undergoing preclinical testing for use as anticancer treatments, which if eventually licensed could be repurposed for fibrotic conditions. Additionally, the glutaminase inhibitor CB-839 is currently being tested in a Phase Ib clinical trial for patients with solid tumours, while the mTOR inhibitor rapamycin is undergoing a Phase II clinical trial for ALS treatment [114]. The AMPK activator metformin has been found to be antifibrotic *in vitro* in lung fibroblasts [115] and a trial is ongoing. Furthermore, a proof-of-concept trial of the inhibition of mTOR/PI3K signalling with omipalisib in pulmonary fibrosis has been completed (NCT01725139). It will be of interest to monitor their performance, which if favourable may pave the way for their use in the treatment of severe fibrotic disorders.

As described, the NAD^+:NADH ratio is regulated by the equilibrium between glycolysis and OXPHOS, mediating many of the effects that occur downstream of metabolic reprogramming. Hence, it is posited here that alteration of the NAD^+/NADH balance contributes to the profibrotic outcome following the glycolytic shift that occurs in fibrosis, with impaired SIRT1 function particularly key. It would therefore also be of significant interest to explore the use of NAD^+-enhancing interventions such as nicotinamide riboside in fibrotic patients and disease models, a therapeutic possibility that has thus far been overlooked. As well as via nicotinamide riboside, the NAD^+-dependent deacetylase SIRT1 can be activated by resveratrol, a well-known dietary supplement consumed with minimal side effects, which also merits further investigation for its antifibrotic potential.

Thus, with the role of altered metabolism becoming increasing accepted as a central mediator of fibrosis, this body of research needs to be translated into new and improved therapeutics. Additionally, the common role of increased glycolysis and glutaminolysis in cancer and fibrosis opens the possibility of utilising breakthroughs in the understanding of cancer metabolism as a means to also gain insight into the potential metabolic mechanisms at play in fibrosis. Given the fact that both glycolysis and glutaminolysis appear to be involved in the disease, combinatorial therapy should be considered. The emergence of itaconate as a key player in the regulation of both inflammation and fibrosis is of great interest. The cell-permeable 4-OI appears to mimic the antifibrotic effect and can do so by upregulating various cytoprotective mechanisms, and requires further investigation.

Outstanding questions

What proportion of cells, immune or stromal, using glycolysis is important in driving fibrosis generation?

Is there a hierarchy in the metabolic pathways mediating fibrosis?

What are the molecular mechanisms pertaining to the effects of the metabolite itaconate?

Is lactylation druggable?

Declaration of interests

No interests are declared.

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