Metabolism Clinical and Experimental 93 (2019) 44–51

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Review

The role of metabolism in the pathogenesis of systemic sclerosis

Honglin Zhu, Weilin Chen, Di Liu, Hui Luo *

Department of Rheumatology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha, Hunan 410008, People's Republic of China

ARTICLE INFO

Article history:
Received 25 September 2018
Accepted 14 December 2018

Keywords:
SSc
Metabolism
Glycolysis
Fatty acid metabolism
Oxidative stress

ABSTRACT

Systemic sclerosis (SSc) is an immune-mediated autoimmune disease characterized by fibrosis and vascular abnormalities. The cellular and molecular mechanisms remain unclear, and current therapies are limited. Cell metabolism has been shown to play an essential role in cancer survival and tumour invasion as well as in rheumatic diseases such as systemic lupus erythematosus, rheumatoid arthritis and osteoarthritis. Although little is known about SSc, cell metabolism may provide new clues for understanding its pathogenesis. In this review, we summarize recent studies of metabolism in SSc and fibrotic disease, specifically focusing on glycolysis, fatty acid metabolism and oxidative stress. We highlight the role of metabolism in fibroblast differentiation and emphasize its potential therapeutic prospects in SSc.

Contents

1. Introduction

Systemic sclerosis (scleroderma, SSc) is an immune-mediated, highly heterogeneous rheumatic disease characterized by fibrosis of the skin and internal organs and vascular abnormalities including Raynaud's phenomenon (RP) and pulmonary artery hypertension [1,2]. The prevalence of SSc is estimated at ~1/10,000 in the general population; however, mortality is highest in those with rheumatic disease [1]. The main cause of death is usually lung fibrosis or pulmonary arterial hypertension. SSc is divided into two clinical subsets based on the extent and severity of skin and organ involvement: diffuse cutaneous SSc (dSSc) and limited cutaneous SSc (lSSc). In dSSc, fibrosis is widespread throughout the skin as well as the visceral organs, while in lSSc, fibrosis is primarily restricted to the hands, arms and face [3]. The mechanism of SSc is very complicated. Self-perpetuating autoimmunity,

* Corresponding author.
E-mail address: luohui@csu.edu.cn (H. Luo).

https://doi.org/10.1016/j.metabol.2018.12.004
0026-0495/

which activates both innate and adaptive immunity, plays a critical role in disease progression and leads to the activation and differentiation of mesenchymal cells and the accumulation of extracellular matrix. Immune cells, platelets, endothelial cells, fibroblasts and many autocrine and paracrine factors all contribute to its pathogenesis [4,5]. Great efforts have been made to elucidate the mechanism of SSc in recent years; however, much remains unknown. Effective treatments are not currently available.

Research into cellular metabolism and immunology has been emerging in recent decades [6–9]. There are six major metabolic pathways: glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), fatty acid oxidation, fatty acid synthesis and amino acid metabolism. Evidence has suggested that tumour cells convert glucose into lactic acid in the presence of oxygen, which is essential for cell survival and invasion (aerobic glycolysis or the Warburg effect) [10]. In addition to tumour cells, many other cell types, such as T cells, macrophages, dendritic cells, myeloid-derived suppressor cells and fibroblasts, also affect metabolic pathways depending on various environmental factors. Different immune cells use distinct metabolic signatures tailored to each biological function. Metabolic reprogramming plays a key role in regulating immune cell function and inflammatory responses and has been studied in rheumatologic diseases [11–16]. In systemic lupus erythematosus, mitochondrial glucose oxidation and hyperpolarization are increased in activated T cells [17]. Normalization of CD4+ T cell metabolism can reverse lupus in a mouse model [18]. In rheumatoid arthritis, decreased glycolysis, glucose shunting into the PPP [19,20] and low ATP levels [21] were observed in naive CD4+ T cells. In fibrotic disease, glycolysis was increased after fibroblast activation [14,15]. However, little is known about cellular metabolism in cases of SSc. Here, we summarize the current understanding of the metabolic aspects of SSc, highlight emerging concepts with potential value for therapy, and discuss the remaining problems to be resolved. This review summarizes new concepts related to metabolism in SSc and new insights for disease therapy in the clinical setting.

2. Altered Metabolism in SSc

Since the 2000s, positron emission tomography (PET) using the glucose analogue tracer ^{18}fluorodeoxyglucose (^{18}F-FDG) has been utilized extensively to detect tumours in the clinical setting. ^{18}F-FDG is dependent on the rate of glycolysis and can be used to quantify glucose uptake [22]. In SSc, PET/CT scans showed increased ^{18}F-FDG uptake in the skin and soft tissue calcinosis [23,24]. SSc patients with interstitial lung disease also demonstrated enhanced ^{18}F-FDG accumulation in the region around the pulmonary lesion, which was positively correlated with disease activity [25]. These results indicated that SSc patients exhibit increased glucose uptake and metabolic reprogramming.

3. Glycolysis

To date, no study has directly investigated the role of glycolysis in SSc. However, research about the role that glycolysis plays in the differentiation of fibroblasts and in fibrotic diseases is rapidly emerging. In the lung tissue of patients with idiopathic pulmonary fibrosis (IPF), mass spectrometry has suggested disruption of amino acid metabolism, glycolysis, the TCA cycle, and mitochondrial beta-oxidation [14,26]. Augmented glycolysis was found in lung fibroblasts isolated from IPF patients and in TGF-β1-treated normal lung fibroblasts. Glycolytic enzymes, including PFKFB3, PFK1 and HK2, were upregulated, and inhibition of PFKFB3 could potentially blunt the differentiation of fibroblasts and attenuate the profibrotic phenotype. Inhibition of glycolysis can decrease the extent of fibrosis in vivo [27]. In bleomycin-induced lung fibrosis mice, increased glycolysis played a critical role in fibroblast differentiation and fibrotic progression, and GLUT1-activated and AMP-activated protein kinase and hypoxia-inducible factor (HIF) 1α/PDK1 participated in fibrotic progression. Inhibition of GLUT1, HIF-1A or PDK1 resulted in a significant reduction in fibrosis [15,28,29]. Increased glycolysis and fatty acid oxidation were also found in the profibrotic M2-like profile of fibrotic lung alveolar macrophages isolated from bleomycin- or active TGF-β1-induced fibrotic mouse lungs [30]. In biopsy samples taken from patients with cirrhosis, carbon tetrachloride (CCl_4) or bile duct ligation induced hepatic fibrosis in mice, and the number of glycolytic stromal cells was positively correlated with the severity of fibrosis. High levels of glycolytic enzymes, such as HK2, PKM2, PFKP, GLUT1, and MCT4, the lipogenic gene peroxisome proliferator-activated receptor γ (PPARγ), and lactate, were present during the transdifferentiation of cultured, quiescent hepatic stellate cells (HSCs) into myofibroblasts. This process is mediated by HIF1α and Hedgehog signalling, which control the fate of HSCs via metabolic regulation. Inhibition of glycolysis, lactate accumulation, Hedgehog signalling or HIF1α can reverse myofibroblasts into quiescent HSCs [31,32]; lactate can also induce collagen expression in baboon liver fibroblasts [33]. High levels of glucose uptake, lactate production and glycolytic enzymes (HK, PKM2, PDK1, and PDK4) were found in unilateral ureter obstruction (UUO)-induced renal fibrosis mice and in TGFβ1-treated renal interstitial fibroblasts. Increasing glycolysis significantly induced the activation of myofibroblasts. Inhibiting glycolysis via 2-deoxyglucose (2DG) and shikonin (reducing PKM2) can reduce mouse renal fibrosis [34]. Taken together, the currently available evidence indicates that glycolysis is critical in fibroblast differentiation and the progression of fibrosis. Multiple interventions can effectively reverse fibrosis both in vitro and in vivo, providing valuable directions for further studies of SSc.

4. Fatty Acid Metabolism

Early in the 1970s, intradermal adipose tissue was found to be atrophied and replaced by collagen-rich fibrous tissue in SSc [35]. Adipocyte progenitor cells, which are essential for limiting fibrosis, are lost during the process of skin fibrosis [36]. Enhanced fibrosis with reduced body fat mass accumulation and small-sized adipocytes were found in lipodystrophy patients and TGF-β1 transgenic fibrosis mice [37,38]. Recently, dermal white adipose tissue (dWAT) was identified as a unique adipose tissue that is distinct from hypodermal adipose tissue and may play a critical role in skin barrier function. dWAT was reduced in SSc patients and fibrosis mice, and this reduction adversely affected skin homeostasis and promoted dermal fibrosis [39]. Improved dWAT loss, dermal fibrosis and survival were observed when fibrosis model mice were injected intradermally with adipose-derived stem cells [36,40]. In addition to dWAT loss, many of the canonical adipogenic markers, including fatty acid-binding protein 4 (FABP4), PPARγ2 and adiponectin, were also reduced in skin lesions. Adiponectin-positive intradermal progenitors differentiated into dermal myofibroblasts [41]. PPARγ is critical for adipocyte differentiation and has proven to be a master regulator of adipogenesis. Pharmacological PPARγ activation in bleomycin-induced skin fibrosis mice promotes systemic adipogenesis and attenuates inflammation and dermal fibrosis [42]. The Wnt/β-catenin pathway, which is critical for fibroblast activation, also disrupts adipocyte differentiation and induces fibrosis [43]. All these studies suggest an important connection between fibrosis and adipose tissue. Thus, fatty acid metabolism might be a contributing factor.

Fatty acids are essential for maintaining cell membrane integrity, signal transduction and energy production and regulating inflammation [44–46]. The monounsaturated free fatty acid oleate can increase the secretion of TGF-β, collagen I, fibronectin, and alpha smooth muscle actin (α-SMA) and induce a myofibroblast phenotype in mesangial cells [47]. In the plasma and red cell membranes of SSc patients, the levels of the prostaglandin precursors dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA) and metabolites of AA (22:4n-6, 22:5n-6), which have important roles in the maintenance of normal cell membrane characteristics, were found to be exceptionally low. Supplementation with essential fatty acids has been found to be beneficial in SSc [48].

Considerable evidence has suggested that fatty acid metabolism is critical in fibrosis and other fibrotic diseases. The inflammatory and metabolic pathways in tubulointerstitial fibrosis patients were significantly altered. Critical enzymes in the regulation of fatty acid oxidation were decreased in tubulointerstitial fibrosis patients and mice, and increased intracellular lipid deposition was also noted. Inhibition of fatty acid oxidation in tubule epithelial cells led to ATP depletion, cell death, dedifferentiation and intracellular lipid deposition, similar to the effects observed in fibrosis. Using genetic or pharmacological methods to restore fatty acid metabolism can protect mice from tubulointerstitial fibrosis [49]. In cystic fibrosis, the release of AA from cell membrane phospholipids was increased, and lower levels of linoleic acid (LA) and docosahexaenoic acid (DHA) and disturbances in annexins and ceramides were described [50]. In porcine aortic valvular interstitial cells, exogenous polyunsaturated fatty acids (PUFAs), DHA and AA were able to reverse the myofibroblastic phenotype mediated by RhoA/G-actin/MRTF signalling [51]. In human prostate carcinoma, DHA inhibited TGF-β-induced myofibroblast differentiation and matrix metalloproteinase-2 (MMP2) production and suppressed the epithelial-mesenchymal transition (EMT) [52]. In cardiac fibroblasts, omega-3 PUFAs [eicosapentaenoic acid (EPA) and DHA] from fish oil blocked TGF-β1-induced phospho-Smad2/3 nuclear translocation through activation of the cyclic GMP/protein kinase G pathway and prevented cardiac fibrosis and dysfunction [53]. Nitrate-derived unsaturated fatty acids display pleiotropic behaviours and are critical in the inhibition of inflammatory cell function [54]. Nitrated fatty acids (NFAs) upregulated PPARγ, converted TGF-β to inactive monomers in a cell-free solution and blocked TGF-β signalling and activity in human lung fibroblasts. NFAs reduced disease severity, reversed myofibroblasts and decreased collagen deposition in an experimental pulmonary fibrosis mouse model [55]. Omega-3 PUFA derivatives, resolvins, have an anti-inflammatory effect by reducing neutrophil infiltration, paw oedema and proinflammatory cytokine expression [56]. Resolvin inhibited PDGF-BB-induced fibroblast proliferation and induced transient activation of the pro-proliferative ERK and AKT signalling pathways; it also exerted direct anti-fibrotic effects on UUO mice [57].

5. TCA Cycle

Intermediate metabolites in the TCA cycle were studied in fibrotic diseases. Succinate, which is formed in the TCA cycle, bound specifically to G protein-coupled receptor 91 (GPR91) and raised the levels of GPR91, α-SMA, TGF-β and type I collagen in HSCs. C57BL6/J mice fed an MCD diet (inhibitor of succinate dehydrogenase, SDH) exhibited elevated plasma succinate levels, increased succinate concentrations, and GPR91 and alpha-SMA expression in isolated HSCs [58]. Succinate was significantly upregulated in lung myofibroblasts and human fibrotic lungs with IPF, which markedly increased TGF-β1-induced HIF-1a expression and promoted fibroblast differentiation [27].

6. Amino Acid Metabolism

The lung consists of active tissue with rapid-turnover metabolism of both collagen and noncollagen proteins. In bleomycin-induced pulmonary fibrosis rabbits, an increased rate of synthesis, a decreased rate of degradation and rapid accumulation of collagen were found in the early stages of pulmonary fibrosis [59]. Genome-wide transcriptome analyses of kidney samples from severe tubulointerstitial fibrosis patients and mice suggested that carbohydrate, amino acid and lipid metabolism were markedly dysregulated [49]. Intracellular glutamine is utilized in the production of biological energy; synthesis of proteins, lipids and nucleotides; and maintenance of redox homeostasis. Glutamine 1 was upregulated in bleomycin-induced lung fibrosis and areas of human IPF. Aberrant glutamine metabolism plays a critical role in lung fibroblast differentiation [60]. Inhibition of glutamine metabolism attenuated bleomycin-induced lung injury, improved survival and diminished fibrosis by reducing Th17 cells and M2 macrophages [61]. An intimate connection between glutamine and Wnt signalling has been found, and modulated glutamine metabolism might provide a novel therapeutic method for SSc fibrosis [62] (Fig. 1).

7. Metabolic Reprogramming in Immune Cells

Each immune cell undergoes specific metabolic reprogramming during activation and differentiation, and these changes are essential for immunological functions. SSc is driven by the activation and effector functions of both innate and adaptive cells. Therefore, metabolic reprogramming in immune cells might play a central role in SSc pathogenesis [63,64].

7.1. T Cells

Activated T cells were found in the peripheral blood and lesional skin tissues of SSc patients, as indicated by increased T cell-derived cytokines in the serum and T cell activation markers in dermal tissues [65]. For CD4+ T cells, the balance of type 1 T helper (Th1)/Th2 cells and regulatory T cells (Tregs)/Th17 cells was disrupted in SSc patients [66,67]. Th1 cells can secrete interferon (IFN)-γ and inhibit collagen production and are decreased in SSc, whereas the Th2 cells are increased and can produce IL-4, IL-5, and IL-13 and promote mononuclear cell infiltration and fibrosis [68]. Increased levels of Th17 cells resulted in the activation of fibroblasts, vascular endothelial cells and smooth muscle cells through IL-17 [69]. Although the percentage of circulating Tregs in SSc patients is controversial, reduced functional capacity has been widely identified. Tregs secrete TGF-β and IL-10, suppress effector T cells and antigen-presenting cell activation, and contribute to the pathogenesis of SSc [70]. Th9 and Th22 cells, which produce IL-9 and IL-22, respectively, were increased in the circulation of SSc patients and were found to be associated with disease activity or complications [71,72]. The role of T follicular helper (Tfh) cells, which secrete IL-21, has not been identified in SSc and needs to be further confirmed. Among CD8 + T cells, the proportions of effector (CD8 + CD45RA + CD27-) and effector/memory (CD8 + CD45RA-CD27-) cells in the peripheral blood are increased. The latter produce IL-13 and IFN-γ and infiltrate the lesional skin in the early stage of dSSc [73]. CD8+ T cells exert proinflammatory and profibrotic activities and induce cytotoxic tissue damage in SSc [74].

Metabolic reprogramming is closely correlated with T cell survival, development, activation, function and differentiation [75]. Naive T cells utilize small amounts of glucose to generate ATP and maintain homeostatic proliferation and survival. The TCA cycle and oxidative phosphorylation are their predominant metabolic pathways [76]. Upon activation, CD4+ T cells differentiate into diverse subsets with distinct metabolic requirements. All subsets rely on glycolysis and oxidative phosphorylation, and Th2 and Th17 cells exhibit even higher levels of glucose uptake [77]. Tregs utilize fatty acid oxidation, while Th17 cells rely on de novo fatty acid synthesis; the balance of fatty acid oxidation and synthesis contributes to the differentiation of Th17 cells and Tregs [78,79]. Upon activation, CD8+ T cells exhibit increases in glycolytic rate and oxidative phosphorylation and differentiate into effector cells. Memory T cells exhibit a decreased glycolytic rate, utilize fatty acid oxidation and have more spare respiratory capacity than naive T cells and thus have a rapid recall capacity upon challenge [75,80].

7.2. B Cells

Hyperactivated B cells were found in SSc patients, as indicated by the overexpression of autoantibodies. B cells drive proinflammatory/profibrotic cytokines, upregulate stimulatory CD19 receptors and impair inhibitory CD22 receptors. The percentage of naive B cells was increased, the level of memory B cells was decreased but the cells themselves markedly activated, and IL-10-producing B regulatory cells

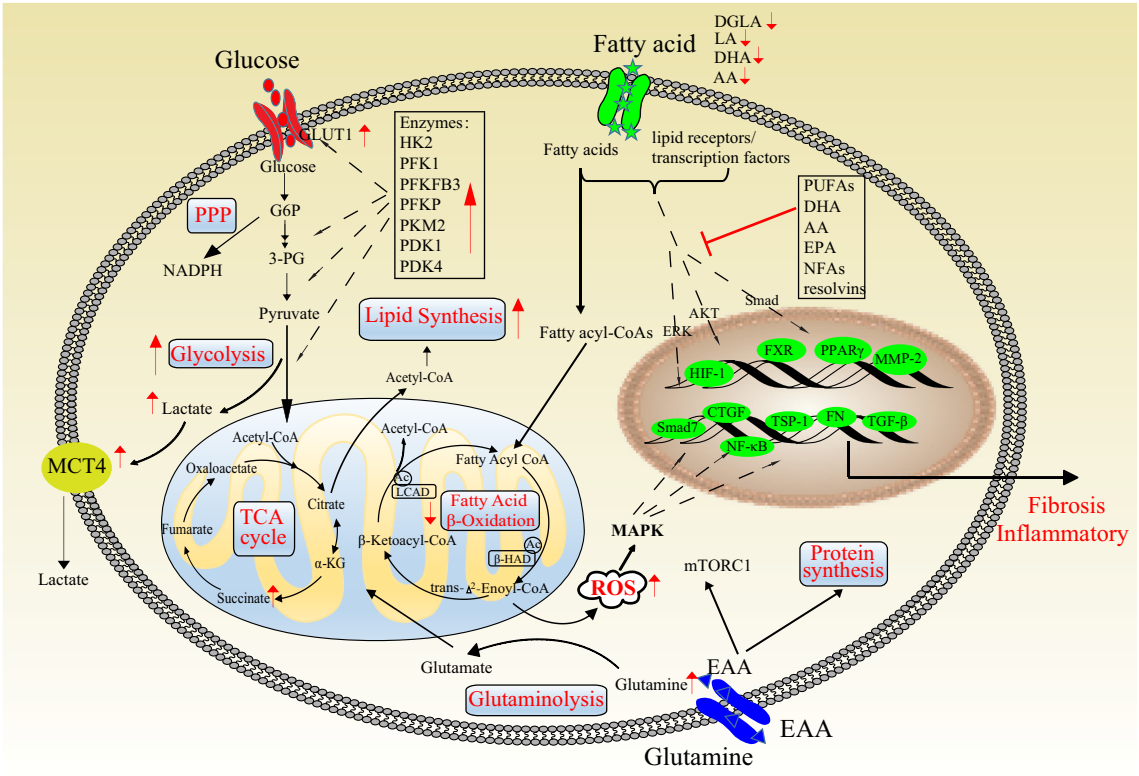

Fig. 1. Dysregulated metabolic pathways in the fibroblast of SSc or fibrotic diseases. Increased glycolysis, TCA cycle, and lipid synthesis and decreased fatty acid β-oxidation are found during fibroblast activation. Multiple essential fatty acid or derivatives (PUFAs, DHA, AA, EPA, NFAs, resolvins) can inhibit fibroblasts proliferation and transdifferentiation. Metabolic reprogramming results in redox imbalance and ROS accumulation, the latter activates multiple signalling pathways and exerts pro-fibrosis and pro-inflammatory effects. HK2, hexokinase 2; GLUT1, glucose transporter 1; PFK-1, phosphofructokinase-1; PFKFB3, fructose-2,6-biphosphatase 3; PFKP, phosphofructokinase; PKM2, pyruvate kinase M 2; PDK1, pyruvate dehydrogenase kinase 1; PDK4, pyruvate dehydrogenase kinase 4; MCT4, monocarboxylate transporter 4; PPP, pentose phosphate pathway; G6P, glucose-6-phosphate; 3-PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; DGLA, dihomo-gamma-linolenic acid; LA, linoleic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; NFAs, nitrated fatty acids; EAAs, essential amino acids; mTORC1, mTOR complex 1.

were reduced in SSc patients. Interestingly, the mechanistic target of rapamycin (mTOR) phosphorylation, which is a central regulator of immunity, was significantly decreased in B cells, suggesting a switch in metabolic pathways in B cells in SSc [81,82].

Different subsets of B cells have different metabolic needs. Upon activation, B cells rapidly increase glucose uptake [6]. High metabolic demands were found in the early proliferative stages of B cells in bone marrow, including the pre-B, immature, and transitional stages of the bone marrow and spleen, and even higher metabolic needs were found in plasma cells that secreted high-affinity antibodies. Energy needs in quiescent blood-borne naive and memory B cells were relatively low but increased during antigen-driven proliferation. The anabolic metabolism was dysregulated in activated B cells, glycolysis and glutaminolysis were increased, and oxidation was decreased. A high level of catabolic metabolism, including fatty acid oxidation and pyruvate oxidation, was found in memory B cells [83,84].

7.3. Other Immune Cells

Soluble CD163, a putative marker of type 2 (M2) macrophages, was increased in the peripheral blood of SSc patients and was correlated with a poor clinical outcome [85]. Abundant M2 macrophages infiltrated the lesional skin and lung tissues of SSc patients [86]. M2 macrophages play key roles in the process of tissue fibrosis. In activated lung-resident macrophages, the expression of lipid and cholesterol trafficking-related genes was increased, indicating metabolic reprogramming in these cells [87].

The frequency of plasmacytoid dendritic cells (pDC) was reduced in the peripheral circulation of SSc patients but increased in the affected skin tissues [86]. CXCL4 secretion by pDCs was significantly increased in the circulation and associated with SSc skin fibrosis and pulmonary arterial hypertension [88].

Elevated numbers of type 2 innate lymphoid cells (ILC2) were found in the peripheral blood and lesional skin of SSc patients, and the number of cells was correlated with the extent of cutaneous fibrosis [89]. Natural killer (NK) cells [90] and neutrophils [91] exhibited altered properties and phenotypes in the blood of SSc patients; however, their roles are unclear.

Overall, both innate and adaptive immune cells display obvious metabolic changes. The six metabolic pathways affect the activation and differentiation of immune cells and might play critical roles in SSc. Extensive research has been conducted to explore metabolic reprogramming under normal conditions. However, little is known about the metabolic changes in immune cells in the pathophysiology of SSc. Further investigations in this field will provide substantial information regarding the mechanism of SSc.

8. Oxidative Stress and SSc

Increasing evidence has indicated the presence of intimate crosstalk between redox balance and metabolism. Metabolic pathways, such as glycolysis, glutaminolysis, fatty acid oxidation, one carbon metabolism and the PPP, can generate the antioxidant molecules NADPH and GSH as well as the redox cofactors NADH and FADH, which are essential for producing and removing reactive oxygen species (ROS) and maintaining redox homeostasis [92,93]. The role of oxidative stress in the pathogenesis of SSc has been widely studied in recent years. In early 1993, Murrel first proposed the important role of oxidative stress in the aetiology of SSc [94]. Subsequently, a series of studies supported this

hypothesis. In SSc, circulating oxidative stress markers, including nitric oxide, malondialdehyde (MDA), asymmetric dimethylarginine (ADMA) and hydroperoxides (ROOHs), were increased, and antioxidant components, such as superoxide dismutase and vitamin C, were decreased [95]. High levels of MDA were negatively correlated with disease duration. Carbonyl was inversely correlated with the modified Rodnan skin score (mRSS) and was decreased in patients with pulmonary fibrosis. ROOHs were positively correlated with the capillaroscopy semiquantitative rating scale score and the rating system for avascular areas [96,97]. The level of ADMA is a reflection of endothelial cell dysfunction and was shown to be a novel and important biomarker for the prediction of cardiovascular events [98]. Elevated serum 8-isoprostane was positively correlated with the severity of pulmonary fibrosis, extent of renal vascular damage and level of immunological abnormalities [99] and was also correlated with the heat shock protein 70 level; the latter was associated with pulmonary fibrosis, skin sclerosis, renal vascular damage, oxidative stress and inflammation [100]. Advanced oxidation protein products (AOPPs), which provide indirect evidence of oxidative stress, were upregulated, stimulating endothelial cells and fibroblasts to produce hydrogen peroxide (H_2O_2), and were involved in vascular or fibrotic complications [101]. Stimulatory PDGFR antibodies were elevated in all SSc patients and appeared to be a specific hallmark. These autoantibodies induced collagen gene expression and fibroblast activation via the Ha-Ras-ERK1/2 and ROS cascades [102]. In SSc skin biopsies, the oxidative stress-related proteins peroxiredoxin 1 and carbonyl reductase I were upregulated, as detected by proteomic analysis [103]. AOPPs were also increased in the skin of dSSc patients, as detected by noninvasive skin autofluorescence [104]. In urine samples, the oxidative production of free radical-catalysed peroxidation of AA, 8-oxo-2′-deoxyguanosine (8-oxodG) and isoprostanes were significantly increased. 8-OxodG was associated with the presence of pulmonary fibrosis and with decreased forced vital capacity and DLCO/alveolar volume. In dSSc, mRSS >14 was independently correlated with the 8-oxodG level [105,106]. Increased levels of 8-isoprostane and H_2O_2 were also found in SSc patients' exhalations [107,108]; however, no correlation with radiological findings was found.

An experimental SSc mouse model also revealed the important roles of oxidative stress. In the tight-skin (TSK-1/+) fibrosis mouse model, the expression levels of the protective antioxidant proteins endothelial nitric oxide synthase (eNOS), haemoxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) in the skin were reduced, although no difference was found in the lung [109]. Overexpressed hydroxyl radicals and superoxide were found in bleomycin-induced mouse fibrosis [110]. More importantly, ROS-inducing agents induced widespread skin and lung fibrosis in BALB/c mice, directly suggesting that ROS play a key role in fibrosis [111]. Oxidative stress and antioxidant molecules are critical for fibroblast activation and function. Therefore, further studies are needed to investigate the major metabolic pathways controlling redox homeostasis in fibroblasts. Interventions targeting these metabolic pathways could facilitate the development of novel antifibrotic therapies (Table 1).

Table 1
Oxidative stress related molecules in SSc.

Sample	Molecules	Reference
SSc circulating	MDA↑	[95]
	Superoxide dismutase↓, vitamin C↓	[95]
	ADMA↑	[95,98]
	ROOHs↑	[95–97]
	8-Isoprostane↑	[99]
	AOPPs↑	[101]
	PDGFR antibodies↑	[103]
Skin biopsies	Peroxiredoxin 1↑, carbonyl reductase I ↑	[103]
	AOPPs ↑	[104]
Urine samples	8-oxodG↑	[105,106]
Exhalations	8-Isoprostane↑, H2O2↑	[107,108]
Skin tissues of tight-skin (TSK-1/+) fibrosis mouse	eNOS↓, HO-1 Nrf2↓	[109]
Bleomycin-induced mouse	Hydroxyl radicals↑, superoxide↑	[110]

9. Conclusion

Given the important roles of metabolism in the activation of immune cells and fibroblasts and in ROS production, the exploitation of metabolic reprogramming interventions may provide attractive and effective therapeutic approaches for SSc. Some targets have been used in clinical practice. The glucose analogue 2-DG and metformin suppressed glycolysis and mitochondrial metabolism. 2-DG plus metformin inhibited the production of cytokines and autoantibodies in lupus-prone B6.sle1.sle2.sle3 mice and normalized the metabolism of SLE CD4+ T cells [18]. PFKFB3 [112] and lactate dehydrogenase (LDH) [113] modulated glycolysis, reduced IFN-γ production and suppressed T cell activation. Targeting glycolysis could inhibit fibroblast differentiation and be beneficial for many fibrotic diseases, as previously mentioned. Methotrexate is an important immune system suppressant and is widely used in rheumatic diseases. Methotrexate can inhibit one carbon metabolism, including amino acid metabolism and nucleotide synthesis, and suppress Janus kinase (JAK)-STAT signalling; it also plays important roles in cell growth, redox balance and epigenetics [114,115]. Due to the critical roles of fatty acid metabolism in SSc, targeting this pathway may be a promising treatment approach. Drugs that modify fatty acid metabolism or dietary supplementation with essential fatty acids could be beneficial in treating SSc.

Regulation of mitochondrial ROS production would also be an interesting target. The mitochondrial ROS scavenger MitoQ can reduce IFN-γ production [116,117]. Pyruvate dehydrogenase kinase 1 (PDHK1) regulates pyruvate flux into the TCA cycle and controls mitochondrial ROS production. Inhibition of PDHK1 promotes an increase in ROS, which induces Tregs and exerts a protective role in experimental autoimmune encephalitis [118].

Metabolic pathway analysis broadens our understanding of the mechanisms of SSc and provides insights into novel therapeutic agents. Each metabolic pathway has specific nutrient transporters, enzymes, and multiple metabolic steps, many of which can be used as targets to design pharmacological intervention.

Over the past decade, considerable evidence has suggested the critical role of metabolism in fibroblast differentiation, fibrotic diseases and SSc. Most studies have focused on glycolysis and fatty acid metabolism as well as oxidative stress and have provided new clues for exploring the mechanisms of SSc. However, immune or inflammatory cells, endothelial cells, and epithelial cells all play important roles in its pathogenesis. In addition to fibroblasts, studies about metabolic reprogramming in immune cells are valuable and urgently needed. Meanwhile, investigations into the TCA cycle, the PPP, and amino acid metabolism are almost non-existent and are urgently needed. Nevertheless, the currently available data have led us to propose that interventions in glycolysis, fatty acid metabolism or oxidative stress could benefit SSc fibrosis. Future research should focus on improving our understanding of metabolic mechanisms in immune cells, fibrosis and vascular abnormalities, on searching for available metabolic intermediates for disease biomarkers, and on identifying the most important enzymes for therapeutic targeting.

Accumulated data have indicated important contributions of metabolism to the pathogenesis of SSc and fibrotic diseases. Multiple approaches are available to restore the dysregulated metabolic pathways, such as 2-DG and metformin inhibit glycolysis, methotrexate suppresses one carbon metabolism, and MitoQ decreases ROS production. Many of them exhibit anti-fibrosis effects in mouse model. Alterations in metabolite levels can affect gene expression and cell signalling. Some metabolites are correlated with SSc activity and the extent of fibrosis. Circulating metabolites have the potential to be novel diagnostic and prognostic biomarkers. However, more works are needed to explore metabolic targets and biomarkers for clinical use.

Author Contributions

HZ wrote first draft. WC and DL revised the manuscript. HL has revised final version and added extra information.

Funding

This study was funded by grants from National Natural Science Foundation of China (81771765) and Hunan Provincial Natural Science Foundation (2018JJ3823).

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The authors thank Pro. Xiaoxia Zuo in Division of Rheumatology, Xiangya Hospital, for providing important suggestions in preparing the manuscript.

References

[1] Denton CP, Khanna D. Systemic sclerosis. Lancet 2017;390:1685–99. https://doi.org/10.1016/S0140-6736(17)30933-9.
[2] Allanore Y, Simms R, Distler O. Systemic sclerosis. Nat Rev Dis Primers 2015;1:15002. https://doi.org/10.1038/nrdp.2015.2.
[3] LeRoy EC, Black C, Fleischmajer R. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202–5.
[4] Gilbane AJ, Denton CP, Holmes AM. Scleroderma pathogenesis: a pivotal role for fibroblasts as effector cells. Arthritis Res Ther 2013;15:215. https://doi.org/10.1186/ar4230.
[5] Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. Nat Rev Rheumatol 2011;8:42–54. https://doi.org/10.1038/nrrheum.2011.149.
[6] Rhoads JP, Major AS, Rathmell JC. Fine tuning of immunometabolism for the treatment of rheumatic diseases. Nat Rev Rheumatol 2017;13:313–20. https://doi.org/10.1038/nrrheum.2017.54.
[7] Perl A. Review: metabolic control of immune system activation in rheumatic diseases. Arthritis Rheumatol 2017;69:2259–70. https://doi.org/10.1002/art.40223.
[8] Kapnick SM, Pacheco SE, McGuire PJ. The emerging role of immune dysfunction in mitochondrial diseases as a paradigm for understanding immunometabolism. Metabolism 2018;81:97–112. https://doi.org/10.1016/j.metabol.2017.11.010.
[9] Peradze N, Farr OM, Mantzoros CS. Research developments in metabolism 2018. Metabolism 2018. https://doi.org/10.1016/j.metabol.2018.11.011.
[10] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33. https://doi.org/10.1126/science.1160809.
[11] Morel L. Immunometabolism in systemic lupus erythematosus. Nat Rev Rheumatol 2017;13:280–90. https://doi.org/10.1038/nrrheum.2017.43.
[12] Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. Nat Rev Rheumatol 2017;13:291–301. https://doi.org/10.1038/nrrheum.2017.49.
[13] Mobasheri A, Rayman MP, Gualillo O. The role of metabolism in the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2017;13:302–11. https://doi.org/10.1038/nrrheum.2017.50.
[14] Zhao YD, Yin L, Archer S. Metabolic heterogeneity of idiopathic pulmonary fibrosis: a metabolomic study. BMJ Open Respir Res 2017;4:e000183. https://doi.org/10.1136/bmjresp-2017-000183.
[15] Zhao H, Dennery PA, Yao H. Metabolic reprogramming in the pathogenesis of chronic lung diseases, including BPD, COPD, and pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2018;314:L544–54. https://doi.org/10.1152/ajplung.00521.2017.
[16] Nwosu ZC, Alborzinia H, Wolfl S. Evolving insights on metabolism, autophagy, and epigenetics in liver myofibroblasts. Front Physiol 2016;7:191. https://doi.org/10.3389/fphys.2016.00191.
[17] Wahl DR, Petersen B, Warner R. Characterization of the metabolic phenotype of chronically activated lymphocytes. Lupus 2010;19(13). https://doi.org/10.1177/0961203310373109.
[18] Yin Y, Choi SC, Xu Z. Normalization of CD4+ T cell metabolism reverses lupus. Sci Transl Med 2015;7(274). https://doi.org/10.1126/scitranslmed.aaa0835.
[19] Yang Z, Y S, Oishi H. Restoring oxidant signaling suppresses proarthritogenic T cell effector functions in rheumatoid arthritis. Sci Transl Med 2016;8(331):331ra38. https://doi.org/10.1126/scitranslmed.aad7151.
[20] Kumar P, Yao LJ, Saidin S. Molecular mechanisms of autophagic memory in pathogenic T cells in human arthritis. J Autoimmun 2018;94:90–8. https://doi.org/10.1016/j.jaut.2018.07.014.
[21] Yang Z, Fujii H, Mohan SV. Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. J Exp Med 2013;210:2119–34. https://doi.org/10.1084/jem.20130252.
[22] Weber WA, Avril N, Schwaiger M. Relevance of positron emission tomography (PET) in oncology. Strahlenther Onkol 1999;175:356–73.
[23] Vadrucci M, Castellani M, Benti R. Active subcutaneous calcinosis demonstrated by fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography in a case of limited cutaneous systemic sclerosis. Indian J Nucl Med 2016;31:154–5. https://doi.org/10.4103/0972-3919.178335.
[24] Oksuzoglu K, Ozen G, Inanir S. Flip-flop phenomenon in systemic sclerosis on fluorodeoxyglucose positron emission tomography/computed tomography. Indian J Nucl Med 2015;30:350–1. https://doi.org/10.4103/0972-3919.164018.
[25] Nishiyama Y, Yamamoto Y, Dobashi H. Clinical value of 18F-fluorodeoxyglucose positron emission tomography in patients with connective tissue disease. Jpn J Radiol 2010;28:405–13. https://doi.org/10.1007/s11604-010-0445-x.
[26] Kang YP, Lee SB, Lee JM. Metabolic profiling regarding pathogenesis of idiopathic pulmonary fibrosis. J Proteome Res 2016;15:1717–24. https://doi.org/10.1021/acs.jproteome.6b00156.
[27] Xie N, Tan Z, Banerjee S. Glycolytic reprogramming in myofibroblast differentiation and lung fibrosis. Am J Respir Crit Care Med 2015;192:1462–74. https://doi.org/10.1164/rccm.201504-0780OC.
[28] Cho SJ, Moon JS, Lee CM. Glucose transporter 1-dependent glycolysis is increased during aging-related lung fibrosis, and phloretin inhibits lung fibrosis. Am J Respir Cell Mol Biol 2017;56:521–31. https://doi.org/10.1165/rcmb.2016-0225OC.
[29] Goodwin J, Choi H, Hsieh MH. Targeting hypoxia-inducible factor-1alpha/pyruvate dehydrogenase kinase 1 Axis by dichloroacetate suppresses bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2018;58:216–31. https://doi.org/10.1165/rcmb.2016-0186OC.
[30] Xie N, Cui H, Ge J. Metabolic characterization and RNA profiling reveal glycolytic dependence of profibrotic phenotype of alveolar macrophages in lung fibrosis. Am J Physiol Lung Cell Mol Physiol 2017;313:L834–44. https://doi.org/10.1152/ajplung.00235.2017.
[31] Lian N, Jiang Y, Zhang F. Curcumin regulates cell fate and metabolism by inhibiting hedgehog signaling in hepatic stellate cells. Lab Invest 2015;95:790–803. https://doi.org/10.1038/labinvest.2015.59.
[32] Chen Y, Choi SS, Michelotti GA. Hedgehog controls hepatic stellate cell fate by regulating metabolism. Gastroenterology 2012;143:1319–29 e11 https://doi.org/10.1053/j.gastro.2012.07.115.
[33] Savolainen ER, Leo MA, Timpl R. Acetaldehyde and lactate stimulate collagen synthesis of cultured baboon liver myofibroblasts. Gastroenterology 1984;87:777–87.
[34] Ding H, Jiang L, Xu J. Inhibiting aerobic glycolysis suppresses renal interstitial fibroblast activation and renal fibrosis. Am J Physiol Renal Physiol 2017;313:F561–75. https://doi.org/10.1152/ajprenal.00036.2017.
[35] Fleischmajer R, Damiano V, Nedwich A. Alteration of subcutaneous tissue in systemic scleroderma. Arch Dermatol 1972;105:59–66.
[36] Chia JJ, Zhu T, Chyou S. Dendritic cells maintain dermal adipose-derived stromal cells in skin fibrosis. J Clin Invest 2016;126:4331–45. https://doi.org/10.1172/JCI85740.
[37] Clouthier DE, Comerford SA, Hammer RE. Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-like syndrome in PEPCK-TGF-beta1 transgenic mice. J Clin Invest 1997;100:2697–713. https://doi.org/10.1172/JCI119815.
[38] McCallum JB, Wu HE, Tang Q. Subtype-specific reduction of voltage-gated calcium current in medium-sized dorsal root ganglion neurons after painful peripheral nerve injury. Neuroscience 2011;179:244–55. https://doi.org/10.1016/j.neuroscience.2011.01.049.
[39] Kasza I, Hernando D, Roldan-Alzate A. Thermogenic profiling using magnetic resonance imaging of dermal and other adipose tissues. JCI Insight 2016;1:e87146. https://doi.org/10.1172/jci.insight.87146.
[40] Varga J, Marangoni RG. Systemic sclerosis in 2016: dermal white adipose tissue implicated in SSc pathogenesis. Nat Rev Rheumatol 2017;13:71–2. https://doi.org/10.1038/nrrheum.2016.223.
[41] Marangoni RG, Korman BD, Wei J. Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. Arthritis Rheumatol 2015;67:1062–73. https://doi.org/10.1002/art.38990.
[42] Wu M, Melichian DS, Chang E. Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. Am J Pathol 2009;174:519–33. https://doi.org/10.2353/ajpath.2009.080574.
[43] Mastrogiannaki M, Lichtenberger BM, Reimer A. beta-Catenin stabilization in skin fibroblasts causes fibrotic lesions by preventing adipocyte differentiation of the reticular dermis. J Invest Dermatol 2016;136:1130–42. https://doi.org/10.1016/j.jid.2016.01.036.
[44] Freigang S, Ampenberger F, Weiss A. Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1alpha and sterile vascular inflammation in atherosclerosis. Nat Immunol 2013;14:1045–53. https://doi.org/10.1038/ni.2704.
[45] Bazinet RP, Laye S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 2014;15:771–85. https://doi.org/10.1038/nrn3820.
[46] Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92–101. https://doi.org/10.1038/nature13479.
[47] Mishra R, Simonson MS. Oleate induces a myofibroblast-like phenotype in mesangial cells. Arterioscler Thromb Vasc Biol 2008;28:541–7. https://doi.org/10.1161/ATVBAHA.107.157339.
[48] Horrobin DF. Essential fatty acid metabolism in diseases of connective tissue with special reference to scleroderma and to Sjogren's syndrome. Med Hypotheses 1984;14:233–47.

[49] Kang HM, Ahn SH, Choi P. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. Nat Med 2015;21:37–46. https://doi.org/10.1038/nm.3762.
[50] Strandvik B. Fatty acid metabolism in cystic fibrosis. Prostaglandins Leukot Essent Fatty Acids 2010;83:121–9. https://doi.org/10.1016/j.plefa.2010.07.002.
[51] Witt W, Buttner P, Jannasch A. Reversal of myofibroblastic activation by polyunsaturated fatty acids in valvular interstitial cells from aortic valves. Role of RhoA/G-actin/MRTF signalling. J Mol Cell Cardiol 2014;74:127–38. https://doi.org/10.1016/j.yjmcc.2014.05.008.
[52] Bianchini F, Giannoni E, Serni S. 22: 6n-3 DHA inhibits differentiation of prostate fibroblasts into myofibroblasts and tumorigenesis. Br J Nutr 2012;108:2129–37. https://doi.org/10.1017/S0007114512000359.
[53] Chen J, Shearer GC, Chen Q. Omega-3 fatty acids prevent pressure overload-induced cardiac fibrosis through activation of cyclic GMP/protein kinase G signaling in cardiac fibroblasts. Circulation 2011;123:584–93. https://doi.org/10.1161/CIRCULATIONAHA.110.971853.
[54] Trostchansky A, Rubbo H. Nitrated fatty acids: mechanisms of formation, chemical characterization, and biological properties. Free Radic Biol Med 2008;44:1887–96. https://doi.org/10.1016/j.freeradbiomed.2008.03.006.
[55] Reddy AT, Lakshmi SP, Zhang Y. Nitrated fatty acids reverse pulmonary fibrosis by dedifferentiating myofibroblasts and promoting collagen uptake by alveolar macrophages. FASEB J 2014;28:5299–310. https://doi.org/10.1096/fj.14-256263.
[56] Xu ZZ, Zhang L, Liu T. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 2010;16:592–7. https://doi.org/10.1038/nm.2123 [1p following 7].
[57] Qu X, Zhang X, Yao J. Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. J Pathol 2012;228:506–19. https://doi.org/10.1002/path.4050.
[58] Li YH, Woo SH, Choi DH. Succinate causes alpha-SMA production through GPR91 activation in hepatic stellate cells. Biochem Biophys Res Commun 2015;463:853–8. https://doi.org/10.1016/j.bbrc.2015.06.023.
[59] Laurent GJ, McAnulty RJ. Protein metabolism during bleomycin-induced pulmonary fibrosis in rabbits. In vivo evidence for collagen accumulation because of increased synthesis and decreased degradation of the newly synthesized collagen. Am Rev Respir Dis 1983;128:82–8. https://doi.org/10.1164/arrd.1983.128.1.82.
[60] Ge J, Xie N, Banerjee S. Metabolic dysregulation of glutamine participates in the pathogenesis of lung fibrosis. Am J Respir Crit Care Med 2017;195:1462–74. https://doi.org/10.1164/rccm.201504-0780OC.
[61] Vigeland CL, Chan-Li Y, Collins SL. Inhibition of glutamine metabolism arrests the development of pulmonary fibrosis. Am J Respir Crit Care Med 2016;193:A4935.
[62] Kordes C, Sawitza I, Haussinger D. Canonical Wnt signaling maintains the quiescent stage of hepatic stellate cells. Biochem Biophys Res Commun 2008;367:116–23. https://doi.org/10.1016/j.bbrc.2007.12.085.
[63] Sakkas LI, Chikanza IC, Platsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. Nat Clin Pract Rheumatol 2006;2:679–85. https://doi.org/10.1038/ncprheum0346.
[64] Laurent P, Sisirak V, Lazaro E. Innate immunity in systemic sclerosis fibrosis: recent advances. Front Immunol 2018;9:1702. https://doi.org/10.3389/fimmu.2018.01702.
[65] Liu M, Wu W, Sun X. New insights into CD4(+) T cell abnormalities in systemic sclerosis. Cytokine Growth Factor Rev 2016;28:31–6. https://doi.org/10.1016/j.cytogfr.2015.12.002.
[66] O'Reilly S, Hugle T, van Laar JM. T cells in systemic sclerosis: a reappraisal. Rheumatology (Oxford) 2012;51:1540–9. https://doi.org/10.1093/rheumatology/kes090.
[67] Fenoglio D, Battaglia F, Parodi A. Alteration of Th17 and Treg cell subpopulations co-exist in patients affected with systemic sclerosis. Clin Immunol 2011;139:249–57. https://doi.org/10.1016/j.clim.2011.01.013.
[68] Fujii H, Hasegawa M, Takehara K. Abnormal expression of intracellular cytokines and chemokine receptors in peripheral blood T lymphocytes from patients with systemic sclerosis. Clin Exp Immunol 2002;130:548–56.
[69] Truchetet ME, Brembilla NC, Montanari E. Interleukin-17A+ cell counts are increased in systemic sclerosis skin and their number is inversely correlated with the extent of skin involvement. Arthritis Rheum 2013;65:1347–56. https://doi.org/10.1002/art.37860.
[70] Slobodin G, Rimar D. Regulatory T cells in systemic sclerosis: a comprehensive review. Clin Rev Allergy Immunol 2017;52:194–201. https://doi.org/10.1007/s12016-016-8563-6.
[71] Yanaba K, Yoshizaki A, Asano Y. Serum interleukin 9 levels are increased in patients with systemic sclerosis: association with lower frequency and severity of pulmonary fibrosis. J Rheumatol 2011;38:2193–7. https://doi.org/10.3899/jrheum.110268.
[72] Mathian A, Parizot C, Dorgham K. Activated and resting regulatory T cell exhaustion concurs with high levels of interleukin-22 expression in systemic sclerosis lesions. Ann Rheum Dis 2012;71:1227–34. https://doi.org/10.1136/annrheumdis-2011-200709.
[73] Takata H, Takiguchi M. Three memory subsets of human CD8+ T cells differently expressing three cytolytic effector molecules. J Immunol 2006;177:4330–40.
[74] Fuschiotti P. Current perspectives on the role of CD8+ T cells in systemic sclerosis. Immunol Lett 2018;195:55–60. https://doi.org/10.1016/j.imlet.2017.10.002.
[75] Geltink Ramon Klein, Kyle Ryan L, Pearce EL. Unraveling the complex interplay between T cell metabolism and function. Annu Rev Immunol 2018;36:461–88. https://doi.org/10.1146/annurev-immunol-042617-053019.
[76] MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. Annu Rev Immunol 2013;31:259–83. https://doi.org/10.1146/annurev-immunol-032712-095956.
[77] Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005;5:844–52. https://doi.org/10.1038/nri1710.
[78] Xu T, Stewart KM, Wang X. Metabolic control of TH17 and induced Treg cell balance by an epigenetic mechanism. Nature 2017;548:228–33. https://doi.org/10.1038/nature23475.
[79] Klysz D, Tai X, Robert PA. Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. Sci Signal 2015;8:ra97. https://doi.org/10.1126/scisignal.aab2610.
[80] Pollizzi KN, Patel CH, Sun IH. mTORC1 and mTORC2 selectively regulate CD8(+) T cell differentiation. J Clin Invest 2015;125:2090–108. https://doi.org/10.1172/JCI77746.
[81] Sakkas LI, Bogdanos DP. Systemic sclerosis: new evidence re-enforces the role of B cells. Autoimmun Rev 2016;15:155–61. https://doi.org/10.1016/j.autrev.2015.10.005.
[82] Forestier A, Guerrier T, Jouvray M. Altered B lymphocyte homeostasis and functions in systemic sclerosis. Autoimmun Rev 2018;17:244–55. https://doi.org/10.1016/j.autrev.2017.10.015.
[83] Boothby M, Rickert RC. Metabolic regulation of the immune humoral response. Immunity 2017;46:743–55. https://doi.org/10.1016/j.immuni.2017.04.009.
[84] Jellusova J, Cato MH, Apgar JR. Gsk3 is a metabolic checkpoint regulator in B cells. Nat Immunol 2017;18:303–12. https://doi.org/10.1038/ni.3664.
[85] Frantz C, Pezet S, Avouac J. Soluble CD163 as a potential biomarker in systemic sclerosis. Dis Markers 2018;2018:8509583. https://doi.org/10.1155/2018/8509583.
[86] Ishikawa O, H. I.. Macrophage infiltration in the skin of patients with systemic sclerosis. J Rheumatol 1992;19(8):1202–6.
[87] Taroni JN, Greene CS, Martyanov V. A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. Genome Med 2017;9:27. https://doi.org/10.1186/s13073-017-0417-1.
[88] van Bon L, Affandi AJ, Broen J. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N Engl J Med 2014;370:433–43. https://doi.org/10.1056/NEJMoa1114576.
[89] Wohlfahrt T, Usherenko S, Englbrecht M. Type 2 innate lymphoid cell counts are increased in patients with systemic sclerosis and correlate with the extent of fibrosis. Ann Rheum Dis 2016;75:623–6. https://doi.org/10.1136/annrheumdis-2015-207388.
[90] Cossu M, van Bon L, Nierkens S. The magnitude of cytokine production by stimulated CD56(+) cells is associated with early stages of systemic sclerosis. Clin Immunol 2016;173:76–80. https://doi.org/10.1016/j.clim.2016.09.004.
[91] Barnes TC, Anderson ME, Edwards SW. Neutrophil-derived reactive oxygen species in SSc. Rheumatology (Oxford) 2012;51:1166–9. https://doi.org/10.1093/rheumatology/ker520.
[92] Panieri E, Santoro MM. ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis 2016;7:e2253. https://doi.org/10.1038/cddis.2016.105.
[93] Smallwood MJ, Nissim A, Knight AR. Oxidative stress in autoimmune rheumatic diseases. Free Radic Biol Med 2018. https://doi.org/10.1016/j.freeradbiomed.2018.05.086.
[94] Murrell DF. A radical proposal for the pathogenesis of scleroderma. J Am Acad Dermatol 1993;28:78–85.
[95] Luo JY, Liu X, Jiang M. Oxidative stress markers in blood in systemic sclerosis: a meta-analysis. Mod Rheumatol 2017;27:306–14. https://doi.org/10.1080/14397595.2016.1206510.
[96] Tikly M, Channa K, Theodorou P. Lipid peroxidation and trace elements in systemic sclerosis. Clin Rheumatol 2006;25:320–4. https://doi.org/10.1007/s10067-005-0013-4.
[97] Riccieri V, Spadaro A, Fuksa L. Specific oxidative stress parameters differently correlate with nailfold capillaroscopy changes and organ involvement in systemic sclerosis. Clin Rheumatol 2008;27:225–30. https://doi.org/10.1007/s10067-007-0769-9.
[98] Boger RH, Maas R, Schulze F. Elevated levels of asymmetric dimethylarginine (ADMA) as a marker of cardiovascular disease and mortality. Clin Chem Lab Med 2005;43:1124–9. https://doi.org/10.1515/CCLM.2005.196.
[99] Ogawa F, Shimizu K, Muroi E. Serum levels of 8-isoprostane, a marker of oxidative stress, are elevated in patients with systemic sclerosis. Rheumatology (Oxford) 2006;45:815–8. https://doi.org/10.1093/rheumatology/kel012.
[100] Ogawa F, Shimizu K, Hara T. Serum levels of heat shock protein 70, a biomarker of cellular stress, are elevated in patients with systemic sclerosis: association with fibrosis and vascular damage. Clin Exp Rheumatol 2008;26:659–62.
[101] Servettaz A, Guilpain P, Goulvestre C. Radical oxygen species production induced by advanced oxidation protein products predicts clinical evolution and response to treatment in systemic sclerosis. Ann Rheum Dis 2007;66:1202–9. https://doi.org/10.1136/ard.2006.067504.
[102] Baroni SS, Santillo M, Bevilacqua F. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006;354:2667–76. https://doi.org/10.1056/NEJMoa052955.
[103] Aden N, Shiwen X, Aden D. Proteomic analysis of scleroderma lesional skin reveals activated wound healing phenotype of epidermal cell layer. Rheumatology (Oxford) 2008;47:1754–60. https://doi.org/10.1093/rheumatology/ken370.
[104] Murray AK, Moore TL, Manning JB. Noninvasive measurement of skin autofluorescence is increased in patients with systemic sclerosis: an indicator of increased advanced glycation endproducts? J Rheumatol 2012;39:1654–8. https://doi.org/10.3899/jrheum.111359.
[105] Avouac J, Borderie D, Ekindjian OG. High DNA oxidative damage in systemic sclerosis. J Rheumatol 2010;37:2540–7. https://doi.org/10.3899/jrheum.100398.
[106] Cracowski JL, Marpeau C, Carpentier PH. Enhanced in vivo lipid peroxidation in scleroderma spectrum disorders. Arthritis Rheum 2001;44:1143–8. https://doi.org/10.1002/1529-0131(200105)44:5<1143::AID-ANR196>3.0.CO;2-#.
[107] Luczynska M, Szkudlarek U, Dziankowska-Bartkowiak B. Elevated exhalation of hydrogen peroxide in patients with systemic sclerosis. Eur J Clin Invest 2003;33:274–9.
[108] Tufvesson E, Bozovic G, Hesselstrand R. Increased cysteinyl-leukotrienes and 8-isoprostane in exhaled breath condensate from systemic sclerosis patients. Rheumatology (Oxford) 2010;49:2322–6. https://doi.org/10.1093/rheumatology/keq271.

[109] Dooley A, Low SY, Holmes A. Nitric oxide synthase expression and activity in the tight-skin mouse model of fibrosis. Rheumatology (Oxford) 2008;47:272–80. https://doi.org/10.1093/rheumatology/kem303.
[110] Oberley LW, Buettner GR. The production of hydroxyl radical by bleomycin and iron (ii). FEBS Lett 1979;97:47–9.
[111] Servettaz A, Goulvestre C, Kavian N. Selective oxidation of DNA topoisomerase 1 induces systemic sclerosis in the mouse. J Immunol 2009;182:5855–64. https://doi.org/10.4049/jimmunol.0803705.
[112] Telang S, Clem BF, Klarer AC. Small molecule inhibition of 6-phosphofructo-2-kinase suppresses T cell activation. J Transl Med 2012;10:95. https://doi.org/10.1186/1479-5876-10-95.
[113] Peng M, Yin N, Chhangawala S. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. Science 2016;354(6311):481–4. https://doi.org/10.1126/science.aaf6284.
[114] Shuvalov O, Petukhov A, Daks A. One-carbon metabolism and nucleotide biosynthesis as attractive targets for anticancer therapy. Oncotarget 2017;8(14):23955–77. https://doi.org/10.18632/oncotarget.15053.
[115] Thomas S, Fisher KH, Snowden JA. Methotrexate is a JAK/STAT pathway inhibitor. PLoS One 2015;10:e0130078. https://doi.org/10.1371/journal.pone.0130078.
[116] Mills EL, Kelly B, Logan A. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 2016;167:457–70 e13 https://doi.org/10.1016/j.cell.2016.08.064.
[117] Buskiewicz IA, Montgomery T, Yasewicz EC. Reactive oxygen species induce virus-independent MAVS oligomerization in systemic lupus erythematosus. Sci Signal 2016;9(456):ra115. https://doi.org/10.1126/scisignal.aaf1933.
[118] Gerriets VA, Kishton RJ, Nichols AG. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J Clin Invest 2015;125:194–207. https://doi.org/10.1172/JCI76012.