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Protein tyrosine phosphatases in systemic sclerosis: potential pathogenic players and therapeutic targets

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Abstract

The pathogenesis of systemic sclerosis depends on a complex interplay between autoimmunity, vasculopathy and fibrosis. Reversible phosphorylation on tyrosine residues in response to growth factors and other stimuli critically regulates each one of these key pathogenic processes. Protein tyrosine kinases, the enzymes that catalyze addition of phosphate to tyrosine residues, are known players in systemic sclerosis, and tyrosine kinases inhibitors are undergoing clinical trials for treatment of this disease. Protein tyrosine phosphatases, the enzymes that counteract the action of tyrosine kinases by removing phosphate from tyrosine residues, are emerging as important signaling mediators and molecular targets for cancer and other common diseases. However, until recently the role of tyrosine phosphatases in systemic sclerosis has remained largely unknown. Here we review the function of tyrosine phosphatases in pathways relevant to the pathogenesis of systemic sclerosis and their potential promise as therapeutic targets to halt progression of this debilitating rheumatic disease.

Keywords

Systemic sclerosis; protein tyrosine phosphorylation; protein tyrosine phosphatase; signal transduction; molecular target

Introduction

Systemic sclerosis (SSc) is a chronic multisystem disorder of the connective tissue, characterized by progressive fibrosis of the skin and internal organs, most commonly the lungs, heart and gastrointestinal tract. SSc is an orphan disease, with a prevalence of up to 75 cases per 100,000 women and remains the connective tissue disease with the highest mortality and morbidity (1).

The pathogenesis of SSc remains incompletely understood. Human genetics and molecular pathology studies in patient samples combined with mechanistic cellular and *in vivo* studies in models of disease have revealed that SSc develops through a unique triad of mechanisms: 1) immunological activation with perivascular infiltration of mononuclear immune cells, 2)

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microvascular endothelial cell activation, and 3) activation of fibroblasts in the skin and organs, which leads to excessive deposition of extracellular matrix and myofibroblast development (2, 3)••. Several of the deregulated signaling pathways that underlie the SSc pathogenic triad (Fig. 1 displays a summary of the pathways that will be considered in this review) –including signaling via toll-like receptors (TLR) (4)•, vascular endothelial growth factor (VEGF) (5)• and the transforming growth factor beta (TGFβ) (6)• just to mention a few- are based on reversible protein tyrosine phosphorylation. The role of protein tyrosine kinases (PTKs) –the enzymes that catalyze the addition of phosphate groups to tyrosine residues– has been extensively studied in different aspects of SSc pathogenesis (7, 8) and inhibitors of PTKs have already entered clinical trials for SSc. For example, a multi-PTK inhibitor known as Nintedanib or BIBF-1120, which is already approved for use in idiopathic pulmonary fibrosis (IPF), and is active in preclinical models of SSc (9) is undergoing a phase III clinical trial in SSc patients with interstitial lung disease (ILD, clinical trial).

On the other hand, the study of protein tyrosine phosphatases (PTPs) -the enzymes that counterbalance PTKs in signal transduction by removing phosphate groups from phosphorylated tyrosine residues- has lagged behind in SSc. However, several recent studies are revealing PTPs as important mediators of signaling pathways that contribute to the triad of pathology that occurs in SSc. (10–12). Here we will highlight recent literature describing the PTPs involved in aspects of the SSc pathogenic triad, emphasizing pathways that are considered of interest for therapeutic purposes.

Classification of PTPs

Dephosphorylation of phosphorylated residues by PTPs is based on the nucleophilic attack of the target phosphate moiety by a critical catalytic cysteine embedded in a conserved C(X)₅R motif (where X could be any amino acidic residue) in the PTP active-site. The C(X)₅R motif defines the PTP family which includes >100 enzymes. Many PTPs –despite the name- can dephosphorylate a variety of phosphorylated moieties in addition to tyrosine, including phospholipids, and are further classified into three distinct classes, based on similarity in amino acidic sequence and tridimensional structure of the catalytic domain (see Table 1 for a simplified scheme of the PTP family). Class I PTPs constitute >95% of all PTPs and are further classified as classical PTPs or dual-specificity PTPs (DSPs). Classical PTPs only dephosphorylate tyrosine residues, and can be classified as transmembrane and non-transmembrane enzymes. There are 21 genes encoding for the former enzymes, which are indicated by the acronym *PTPR* followed by progressive letters. For example, a well-known transmembrane class I PTP is the cluster of differentiation 45 (CD45, encoded by *PTPRC*), which is expressed at high levels on almost all blood leukocytes. The remaining 17 classical PTPs are non-transmembrane enzymes and their gene names are indicated by the acronym *PTPN* followed by progressive numbers. The majority of these enzymes include special domains that recruit the enzymes to specific subcellular domains or protein complexes. For example, two well-known non-transmembrane class I PTPs are proto-oncogene tyrosine protein kinase Src (SRC) homology 2 (SH2)-containing PTP1 and PTP2 (SHP1 and SHP2, encoded by the *PTPN6* and *PTPN11* genes, respectively), which are characterized by two N-terminal SH2 domains that confer the ability to interact with

tyrosine phosphorylated motifs. DSPs are a large subclass of enzymes that can dephosphorylate tyrosine as well as serine, threonine and other phosphorylated moieties. DSPs include typical DSPs as well as other enzymes that have special modes of regulation or can dephosphorylate additional phosphorylated moieties such as phospholipids. Well-known typical DSPs are the mitogen-activated protein kinase (MAPK) phosphatases (MKPs) that dephosphorylate the activation motif of MAPKs (see below for further discussion regarding MAPKs). Class II includes only the tyrosine-specific low molecular weight PTP (LMPTP, encoded by the *ACPI* gene), while Class III includes three cell division cycle 25 (CDC25) enzymes that can dephosphorylate tyrosine and threonine residues and are involved in cell cycle regulation (13). For the purpose of this review, we will focus on the role of classical PTPs which, due to their multi-domain structure, tend to undergo selective involvement in certain signaling pathways. Due to the widespread activation of MAP kinases during cellular signaling, typical DSPs are likely to be involved in all aspects of SSc pathogenesis, however data directly relevant to SSc is currently only available for MAPK phosphatase 1 (MKP1, encoded by the *DUSP1* gene). Very little SSc-relevant information has been reported for other DSPs, Class II or Class III PTPs. The reader interested in learning more about the biochemistry of PTPs or the role of DSPs in MAPK signaling, is referred to the following excellent reviews (14–19).

PTP-mediated regulation of pathogenic pathways underlying immune dysregulation in SSc

An essential step in the pathogenesis of SSc is the abnormal activation of both cellular and humoral arms of the immune system. This step depends on loss of immune tolerance and manifests clinically with skin inflammation and pathologically with perivascular infiltrates of immune cells. Although clinically the inflammatory phase of SSc occurs in the early stage of disease, it is believed that a continuously abnormal immune response contributes to the persistence of vasculopathy and the fibrogenesis that characterize the later stages of disease (20). Immune interventions that are being explored for therapy of SSc include neutralization of several T cell-derived cytokines, and interference with signaling via the TLRs. Here we will briefly review the role of PTPs in signaling by interleukins 4, 6 and 13 (IL-4, IL-6 and IL-13) and by engagement of TLR. We will also review PTPs that regulate signaling pathways that are activated by intravenous immunoglobulins (IVIG), since there is increasing interest in IVIG as potential therapeutics for SSc.

IL-4 and IL-13 receptors

The inflammatory infiltrate of SSc mainly includes macrophages and T lymphocytes. T cells in SSc display increased expression of activation markers, show signs of antigen-driven expansion and produce multiple cytokines (21). Among T-cell derived cytokines, IL-4 and IL-13 are mainly secreted by activated Th2 cells and prominently expressed in SSc (22). Although there is currently no active trial of anti-IL-4 and/or IL-13 in SSc, a phase II study to assess the efficacy of lebrikizumab, a humanized monoclonal antibody blocking IL-13, in patients with IPF, is currently recruiting participants (). IL-4 signals through type I and type II IL-4 receptors (IL-4R), which are heterodimers of the IL-4Ra combined with the IL-13 receptor α 1 (IL-13Ra1) and with the common γ (γ c) chains. Upon engagement of its

receptors, IL-4 activates the two Janus PTKs JAK1 and JAK3 and the downstream signal transducer and activator of transcription protein 6 (STAT6), which binds DNA and regulates gene transcription. IL-4 also promotes tyrosine phosphorylation of the insulin receptor substrate proteins (IRS) 1 and 2. IRS1 and IRS2 activate downstream players including the phosphatidylinositol 3-kinase (PI3K), which leads to activation of protein kinase B (PKB), also known as AKR thymoma (AKT), or the small GTPase rat sarcoma (RAS), which instead leads to activation of MAPKs (23). MAPKs are activated via phosphorylation by MAP kinase kinases, for example the dual specificity mitogen-activated protein kinase kinase 1 (MAP2K1), which in turn are activated by upstream MAP kinase kinase kinases, for example the rapidly accelerated fibrosarcoma (RAF) kinase. Activated MAPKs, for example the extracellular signal-regulated kinase (ERK), are able to shuttle into the nucleus and control gene transcription (24). IL-13 binds the heterodimer between the IL4Ra and IL-13 receptor α 2 (IL-13Ra2), activating the non-receptor tyrosine kinase 2 (TYK2), JAK1 and JAK2, which in turn activate STAT3 and STAT6 (25).

Several PTPs are involved in IL-4 or IL-13 signaling. For example, during IL-4 signaling, **SHP2** (encoded by *PTPN11*) is recruited by IL-4R/JAK, where it dephosphorylates and inactivates STAT6 in mouse peritoneal and alveolar macrophages (26). Additionally, **SHP1** (encoded by *PTPN6*) is implicated in negative regulation of IL-4 and IL-13-dependent activation of STAT6 in fibroblasts and bone marrow-derived macrophages (27). More recently, experiments in pancreatic cells have identified STAT6 as the substrate of other PTPs, including PTP basophil-like (**PTPBL**, encoded by *PTPN13*) and T cell PTP (**TCPTP**, encoded by *PTPN2*), which antagonize STAT6 phosphorylation (28). Another study showed that the protein tyrosine phosphatase PEST (**PTPEST**, encoded by the *PTPN12* gene) enhances IL-4 and IL-13 transcription after Fc ϵ RI crosslinking in cancerous basophil cells (29).

IL-6 receptor

Active SSc associates with elevation of several acute phase reactants, including C-reactive protein and interleukin-6 (IL-6) (30). IL-6 is a pro-inflammatory cytokine synthesized by a wide range of cells, including macrophages and fibroblasts, and is considered a critical factor in SSc pathogenesis. IL-6 levels are elevated in the serum and skin of early SSc patients and, in general they correlate with higher mortality, severe skin involvement and pulmonary failure (31). Tocilizumab is a humanized monoclonal antibody that blocks the IL-6R and has been approved for the treatment of several rheumatic diseases, including rheumatoid arthritis (RA). Treatment with tocilizumab led to a mild improvement of skin thickness and delayed the deterioration of lung functions in a phase II randomized trial in SSc patients (32) prompting an ongoing phase III trial (. The IL-6 receptor (IL-6R), which has transmembrane (tIL-6R) and soluble (sIL-6R) isoforms, requires dimerization with two glycoprotein130 (gp130) subunits to activate downstream signal transduction. IL-6 signaling involves activation of JAK1 and JAK2, with subsequent triggering of STAT1 and STAT3, the PI3K and MAPKs.

SHP1 directly associates with and dephosphorylates JAKs, resulting in deactivation of gp130 signal transduction (33). Transmembrane **PTPEpsilon**, an enzyme encoded by the

PTPRE gene, has a cytosolic isoform that selectively inhibits IL-6-induced JAK-STAT signaling in murine myeloid cells, leading to suppression of STAT3 tyrosine phosphorylation (34). Another study performed in HEK 293 epithelial cells showed that **TCPTP** also negatively regulates IL-6 signaling pathway through STAT3 dephosphorylation (35). Interestingly, myeloma cells require **CD45** (a transmembrane PTP encoded by **PTPRC** gene) to initiate IL-6 signaling and to maintain the activity of LCK/YES novel tyrosine kinase (LYN), a SRC family kinase that is essential for cell proliferation and cell adhesion (36). Additionally, the non-receptor lymphoid tyrosine phosphatase (**LYP**), encoded by the **PTPN22** gene, has been shown to inhibit IL-6R-mediated signaling by blocking the phosphorylation of STAT3 in B cells (37).

Toll-like receptors

Recent studies have highlighted the role of innate immune signaling via TLRs and their endogenous ligands in SSc (4)•. TLRs are transmembrane cellular sensors composed of extracellular leucine-rich repeats, which mediate endogenous and exogenous ligand recognition, and transmembrane Toll/IL-1 receptor (TIR) domains, responsible for intracellular signal transduction. There are many different TLRs: TLR2, TLR4, TLR5, and TLR6 are localized at the plasma membrane, while TLR3, TLR7, TLR8, and TLR9 can be found in intracellular vesicles. SSc patients show elevated levels of TLR4 and of endogenous damage-associated ligands generated by oxidative stress and tissue injury. A proposed approach to SSc treatment consists in the disruption of TLR4 signaling, targeting either the receptor itself or its ligands. Multiple therapeutic strategies are being explored including antibodies, oligodeoxyribonucleotides and small molecules (38). When engaged, TLR4 interacts with the adapter protein myeloid differentiation primary response gene 88 (MyD88) to recruit IL-1 receptor-associated serine/threonine kinase 1 (IRAK1), leading to activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which is able to migrate to the nucleus and regulate transcription of pro-inflammatory genes. In addition, TLR4 can activate a MyD88-independent signaling, which includes the TNF Receptor Associated Factor 3 (TRAF3) and the TANK-binding serine/threonine kinase 1 (TBK1), and culminates in activation of interferon regulatory factor 3 (IRF3), which migrates to the nucleus and induces type I interferon production (39).

The PTP **LYP**, encoded by the **PTPN22** gene, recently emerged as a potential regulator of TLR signaling. A missense polymorphism in **PTPN22** results in the LYP-R620W variation, which is significantly associated with SSc in Caucasian and Afro-American populations. LYP-W620 is also a strong risk factor for RA and systemic lupus erythematosus (40). Both promotion and inhibition of TLR signaling by LYP has been reported via cellular and *in vivo* studies (41, 42). LYP also plays a known role in T cell signaling regulation through dephosphorylation of several PTKs. **PTPPEST** has also been reported to inhibit macrophage TLR signaling in a phosphatase activity-dependent manner by inhibition of I κ B kinase β (IKK β), which is an important promoter of NF- κ B activation (43). **CD45** exerts a complex effect on TLR signaling. The current hypothesis is that CD45 has a negative effect on MyD88-dependent signaling, leading to pro-inflammatory cytokine production, and a positive effect on MyD88-independent signaling, leading to interferon β (IFN β) production (44).

IVIG

IVIG are purified immunoglobulin G (IgG) obtained from pooled human plasma with small trace amounts of immunoglobulin A (IgA) or immunoglobulin M (IgM). IVIG have multiple immune modulatory and anti-inflammatory actions (45). A large number of studies support the usefulness of IVIG in SSc (46) and a clinical trial () is currently validating the effects of Privigen (IVIG) on skin fibrosis in SSc. The Fc gamma receptors (Fc γ R) that recognizes the IgG Fc region of IgG play an important role in SSc inflammation and mediate at least part of the therapeutic action of IVIG. Physiologically, immune complexes, consisting of IgG-covered particles, induce crosslinking of Fc γ R and the subsequent phosphorylation of its immune-receptor tyrosine-based activation motif (ITAM) domains (47). Phosphorylation of the ITAM tyrosine residues induces the activation of SRC and spleen tyrosine kinase (SYK) family PTKs, with sequential activation of PI3K, the MAPKs and of the focal adhesion kinase (FAK), which regulate actin polymerization, phagocytosis, and Fc γ R internalization (48, 49).

SHP1 is involved in the downregulation of Fc γ R signaling in monkey kidney fibroblasts by the dephosphorylation of SYK (50), while **SHP2** seems to increase macrophage Fc γ R-mediated responses, including phagocytosis, through PI3K and MAPKs activation (51). In addition, **CD45** regulates antigen-triggered FcR signaling in mast cells by modulating SRC family kinase activity (44). PTPs can also control Fc γ R signal transduction via ITAM dephosphorylation. The Class I megakaryocyte PTP (**PTPMEG**, encoded by the **PTPN4** gene) and the Class II **LMPTP** (encoded by the **ACPI** gene) are involved in suppression of cell activation mediated by ITAM-associated immune-receptors in T cells and platelets, respectively (52, 53).

PTP-mediated regulation of pathogenic pathways underlying endothelial dysfunction in SSc

Maintenance of the physiologic small vessel tone depends on the coordinate action of endothelial cells, smooth muscle cells, the sympathetic nervous system and multiple soluble mediators. Injury and activation of endothelial cells is an early event in the pathogenesis of SSc. The resulting vasculopathy manifests in the skin as Raynaud's phenomenon (54), which represents an uncontrolled contractile response of small vessels to rapid cooling and is known to correlate with altered PTK activity in the involved vessels (55). Pulmonary arterial hypertension (PAH) is one of the most serious complications of SSc and is caused by functional and structural alterations in the pulmonary arterioles (56). Here we will briefly review the role of PTPs in endothelial signaling via VEGF, a fundamental regulator of angiogenesis, and the receptors for prostacyclins and endothelins, which are the targets of currently used anti-PAH agents in SSc.

VEGF

VEGF is a potent angiogenic growth factor that operates by binding to VEGF receptors (VEGFRs), which function as PTKs. SSc patients show elevated levels of VEGF in affected skin and serum, and increased VEGFRs expression is observed in SSc patients skin throughout different disease stages (57). Several PTK inhibitors, which antagonize VEGFRs

such as lenvatinib (58) or motesanib (59), are approved or under investigation for anti-angiogenic therapy in cancer. VEGF binds to 3 receptors, VEGFR1, VEGFR2 and VEGFR3, which are expressed on several cell populations besides endothelial cells (60). Of these receptors, engagement of VEGFR2, also known as kinase insert domain receptor (KDR) or fetal liver kinase 1 (FLK1), results in activation of protein kinase C (PKC), which promotes activation of MAPKs and DNA synthesis in endothelial cells (61). VEGFR2 also promotes actin reorganization through activation of the SRC-FAK pathway (62).

The non-receptor classical PTP **PTP1B** (encoded by the *PTPNI* gene) is well-expressed in endothelial cells and endothelial-specific PTP1B deletion in mice leads to increased angiogenesis and arteriogenesis through MAPKs signaling (63). The transmembrane PTP **RPTP β** , also known as vascular endothelial-protein tyrosine phosphatase (**VEPTP**, encoded by the *PTPRB* gene), has been shown to dephosphorylate angiopoietin-1 receptor tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (TIE2), a VEGFR downstream modulator responsible for maintaining vascular integrity. VEPTP blocking small molecule AKB-9778 improves endothelial barrier function, restoring TIE2 signaling (64). Thus, targeting VEPTP could pave the way for a potential therapeutic strategy for VEGF signaling modulation in SSc. The megakaryocyte protein tyrosine phosphatase 2 (**PTPMEG2**, encoded by the *PTPN9* gene), has been shown to negatively regulate cytokine production in endothelial cells via dephosphorylation of VEGFR2 (65). Both **SHP1** and **SHP2** have been shown to interact with VEGFR2, suggesting a regulatory function in VEGFR signaling. TNF α enhances **SHP1** recruitment with resulting inhibition of VEGFR2-mediated MAPKs signaling (66), while the role of **SHP2** is incompletely understood, since it does not appear to alter the VEGFR2-phosphorylation (67). Another PTP involved in regulation of VEGF-signaling in endothelial cells is **LMPTP**, whose overexpression attenuated VEGFR2 activation and downstream angiogenesis signaling in rat aortic rings (68).

Prostacyclins

Prostacyclins are potent lipid vasodilators that are used in the clinic for therapy for PAH and bind to the prostacyclin receptor (PTGIR) -a G-protein coupled receptor- on the surface of endothelium and smooth muscle cells. When engaged, PTGIR modulates MAPKs activation through protein kinase A (PKA) activation (69). A complex of **SHP1** and the scaffold protein spinophilin reduces PTGIR response in platelets by retaining and/or releasing members of the regulator of G protein signaling family, such as RGS10 and RGS18 (70).

Endothelins

The endothelins are small peptides, produced mainly by endothelial cells, which regulate vascular tone via binding to G protein-coupled endothelin receptors ETa and ETb. Endothelin-1 (ET-1) is the predominant regulator of vascular tone and binds ETa on muscle cells and ETb on endothelial cells (71). There are currently 3 Food and Drug Administration (FDA) approved endothelin receptor agonists -ETa agonist ambrisentan and ETa/ETb agonists bosentan and macitentan- with proven beneficial effects in patients with PAH. These agents block the vasoconstrictor effects of ETa receptors while maintaining the vasodilator functions of ETb receptors (72, 73). Signaling via ETa involves the activation of MAPKs (74), while activation of ETb leads to recruitment and stimulation of the PI3K-AKT

pathway, resulting in subsequent vasodilator production of nitric oxide (75). Although **SHP2** promotes the activation of some pathways -such as the RAS-MAPK pathway- downstream ETa, overall SHP2 has been shown to negatively regulate ETa signaling. In rat cardiac fibroblasts, ROS generation after ETa stimulation transiently inhibits SHP2 activity via oxidation of the catalytic cysteine to enhance ETa signaling (76). Interesting, a similar positive feedback mechanism mediated by a different PTP has been described for ETa signaling in rabbit platelets where ET-1 induces tyrosine phosphorylation and inactivation of **SHP1** (77).

PTP-mediated regulation of pathogenic pathways underlying fibrosis in SSc

The fibrosis process in SSc consists of pathological deposition of extracellular matrix (ECM) in organs and tissues. This compromises physiological tissue architecture and organ functions, resulting in the major causes of morbidity and mortality in SSc. A key pathogenic feature of fibrosis is represented by the persistent activation of interstitial fibroblasts, which show abnormal phenotype, including increased proliferation, migration, ECM production and resistance to apoptosis. The differentiation of fibroblasts into myofibroblasts is another crucial hallmark of SSc fibrosis. Myofibroblasts express α -smooth muscle actin (α SMA) in stress fibers, have an increased capacity to synthesize collagen and ECM, and are a major source of TGF β (78). Recent studies have revealed that activated myofibroblasts can be formed from endothelial cells, through a process known as endothelial to mesenchymal transition (EMT) (56). Here we will review the role of PTPs in the key pro-fibrotic pathways that are currently targets of anti-fibrotic therapies in SSc –the pathways downstream the receptors for the growth factors TGF β , platelet-derived growth factor (PDGF) and connective tissue growth factor (CTGF), and for lysophosphatidic acid (LPA).

TGF β

Several lines of evidence define the multifunctional cytokine/growth factor TGF β as a central promoter of SSc pathogenesis. SSc patients show increased expression of all three isoforms of TGF β (TGF β 1, TGF β 2 and TGF β 3) in the skin, and high levels of active TGF β are secreted by SSc peripheral blood mononuclear cells (PBMCs). Furthermore, several hallmarks of TGF β activity, such as the presence of myofibroblasts and the expression of ECM proteins, are found in SSc skin and lungs (79)••. Several pre-clinical studies support the anti-fibrotic efficacy of antibodies against TGF β or interference with downstream TGF β signaling (80), however, an initial clinical trial using the anti-TGF β 1 monoclonal antibody metelimumab, also known as CAT-192, did not show efficacy in patients with SSc (81). A possible explanation of this unexpected result is that metelimumab does not block all three isoforms of TGF β . A more recent clinical trial in patients with early diffuse cutaneous SSc showed that fresolimumab, a human monoclonal antibody that binds to and inhibits all three TGF β isoforms, reversed markers of skin fibrosis through inhibition of TGF β -regulated gene expression (82)•. The three TGF β isoforms are encoded by different genes (*TGFB1*, *TGFB2* and *TGFB3*), and interact with heterodimers of TGF β receptor type-1 (TGFR1), also known as activin A receptor type II-like kinase of 53 kDa (ALK5), and TGF β receptor type-2 (TGFR2). Canonical TGF β signaling proceeds through phosphorylation of Sma and

mothers against decapentaplegic homologue 2/3 (SMAD2/3) which enter the nucleus and binds DNA to promote gene transcription (6). Furthermore, TGF β displays several non-canonical, or non-SMAD-mediated, intracellular signaling pathways, including activation of the MAPKs ERK and c-Jun amino terminal kinase (JNK), IKK β , PI3K/AKT and Rho-associated protein kinase (ROCK) to modulate a multitude of downstream cellular responses (83).

A report by the Trojanovska group revealed that levels of the non-typical DSP phosphatase and tensin homolog (**PTEN**) were reduced in skin fibroblasts of SSc subjects. This seminal study led to the first report focused on PTPs in SSc (84). Further experiments showed that mice lacking PTEN displayed an increase in dermal thickness caused by aberrant deposition of collagen. Conversely, overexpression of PTEN in SSc skin fibroblasts reduced the overexpression of type I collagen by inhibition of the PI3K/AKT pathway, thus highlighting the role of PTEN as a critical negative regulator of non-canonical TGF β pro-fibrotic signaling (12). **SHP2**, a known promoter of MAPKs signaling, enhanced TGF β -driven EMT through its phosphatase activity by binding to the adaptor protein GRB2-associated binding protein-1 (GAB1), an upstream modulator of MAPKs, in carcinoma cell lines (85). However, it remains unclear whether SHP2 is a pro-fibrotic phosphatase *in vivo*. A recent study shows that inhibition of SHP2 catalytic activity leads to enhanced murine lung fibrosis in a bleomycin murine model, whereas SHP2 overexpression rescues bleomycin-induced pulmonary fibrosis (86). The mechanism of bleomycin-induced fibrosis includes oxidative damage via oxidant-mediated DNA breaks, causing inflammatory reactions in the lung and skin. The transmembrane PTP **RPTP α** (encoded by the **PTPRA** gene) is a promoter of profibrotic TGF β signaling. Similar to CD45, RPTP α dephosphorylates the inhibitory C-terminal regulatory domain of SRC PTKs, leading to kinase activation. *In vitro* experiments carried out on **Ptpra** KO lung mouse fibroblasts showed attenuated TGF β -induced SMAD transcriptional activity (11). A study on kidney fibrosis also demonstrated that **TCPTP** is an inhibitor of profibrotic TGF β signaling via direct dephosphorylation of TGFR2. Impaired dephosphorylation of TGFR2, in animals carrying *Tcptp* deletion, results into severe kidney damage in a mouse model of renal fibrosis (87).

PDGF

Another important growth factor that stimulates fibrosis in SSc is the platelet-derived growth factor (PDGF). Multiple isoforms of PDGF (PDGF-AA, BB, AB, CC, and DD) are secreted by platelets, monocytes and vascular endothelial cells and bind to the PDGF receptor (PDGFR) isoforms, PDGFR α (or CD140a) and PDGFR β (or CD140b), which have PTK activity (88). SSc patients show higher levels of PDGF and PDGFRs in fibrotic skin lesions (89) and bronchoalveolar lavages (90). Nilotinib is an FDA-approved PTK inhibitor used for the treatment of leukemia that has antagonistic activity against the PDGFR and other PTKs. Recently, an open-label pilot trial confirmed a significant improvement of the modified Rodnan skin score (mRSS) in patients with diffuse SSc treated with Nilotinib (91). In the presence of PDGF, the PDGFR undergoes homo- or hetero-dimerization to activate multiple downstream signaling players, including SRC, MAPKs, PI3K, and PKC (92). Negative regulation of the PDGFR mainly occurs via receptor dephosphorylation rather than internalization or ubiquitin-mediated degradation, suggesting a major role for PTPs in tuning

PDGF signaling (93). An in-gel immunoprecipitation assay to determine the association of PTPs with PDGFR β showed several candidates in HEK 293 epithelial cells, including **PTP1B**, **TCPTP** and **SHP2** (94). PTP1B is the only PTP besides PTEN that has been object of a specific study in SSc. Tsou *et al.* reported that increased levels of ROS in SSc skin cause oxidative inactivation of PTP1B in dermal fibroblasts, thus enhancing PDGFR phosphorylation and promoting collagen synthesis (10, 95). TCPTP has been shown to selectively regulate PDGFR β trafficking in mouse embryonic fibroblasts by inducing receptor recycling without affecting its internalization rate (96). TCPTP and PTP1B regulate the phosphorylation of distinct tyrosine residues of the PDGFR, exemplifying how multiple PTPs need to work in concert to regulate multi-phosphorylated signaling mediators. Experiments on porcine aortic endothelial (PAE) cells indicated **SHP2** as a major promoter of PDGFR β signaling. PDGFR β -mutant PAE cells, lacking the SHP2 binding site, showed impaired migration after PDGF stimulation. Downstream PDGFR β , SHP2 regulates FAK and the PI3K-AKT pathway (97). **LMPTP** and the receptor PTP type J (**DEP1**, also called **CD148** and encoded by the *PTPRJ* gene), are also able to directly dephosphorylate PDGFR and inhibit its mitogenic effects (98, 99). Furthermore, the non-receptor PTP histidine domain-containing PTP (**HDPTP**, encoded by the *PTPN23* gene), promotes the ubiquitination of PDGFR β . Consistently, HDPTP-depleted mouse fibroblasts display faster migration and an increased growth driven by autocrine PDGF (100).

CTGF

The multi-modular protein CTGF, also known as CCN2, is a key pathogenic mediator in SSc. TGF β strongly induces CTGF expression through the canonical SMAD pathway (101). CTGF expression has been found to be enhanced in human diseases characterized by excessive scarring and fibrosis, including PAH and SSc (102). The human anti-CTGF antibody FG-3019 neutralizes CTGF and was first successfully tested in a bleomycin-induced animal model of fibrosis (103) and then proceeded to open-label phase II trial in patients with IPF. The study showed encouraging changes in pulmonary function correlated with amelioration of pulmonary fibrosis in treated patients (104). CTGF interacts with different molecules such as cytokines, growth factors, receptors and matrix proteins to modulate multiple biological functions. In addition, VEGF, integrins, proteoglycans and low-density lipoproteins are targeted by CTGF as well (105). The resulting intracellular signaling is mediated by activation of MAPKs, FAK and the PI3K/AKT pathway (106). CTGF-mediated focal adhesion disassembly in mesangial cells involves dephosphorylation of FAK through increased activity of **SHP2** (107). In the same cell type, it was also shown that CTGF also up-regulates MAPK phosphatase 1 (**MKP1**, encoded by the *DUSP1* gene) expression to promote cell survival (108).

LPA

LPA is a small bioactive glycerophospholipid mainly produced by activated platelets in serum and binds to at least 6 known G-protein-coupled receptors, known as LPA1–6, and one nuclear receptor, the peroxisome proliferator-activated receptor- γ (PPAR γ). LPA levels are significantly higher in serum of SSc patients compared with healthy controls (109). In a pre-clinical lung bleomycin model, the absence of LPA1 in mice led to reduced fibroblast recruitment, vascular leak and decreased collagen deposition (110). The LPA1 antagonist

SAR100842 was administered to diffuse SSc patients in a phase II clinical trial () and resulted in an improvement of the mRSS. Another LPA1 antagonist in clinical trial is BMS-986020, also known as AMS152, which has completed a phase II clinical trial for the treatment of IPF () although the results have not yet been released. LPA receptors vary widely in their structure, tissue distribution and downstream effects (111). Activation of LPA1 modulates the MAPK, PI3K/AKT, and ROCK pathways (112). Moreover, LPA signaling controls EMT through regulation of complexes between the intracellular portion of cadherins –which are transmembrane adhesion molecules- and β -catenin, a key intracellular signaling regulator (113). Studies on ovarian cancer cells showed a role for **PTP1B** in adherens junction stabilization by modulation of β -catenin tyrosine phosphorylation. However, the mechanism of PTP1B in LPA signaling remains largely unknown (114). **SHP1** has also been shown to inhibit tyrosine phosphorylation of G α -interacting vesicle-associated protein (GIV), an enhancer of PI3K/AKT, upon activation of the LPA receptor (115).

Conclusions and translational perspectives

In conclusion, reversible tyrosine phosphorylation is heavily involved in all aspects of the pathogenic triad of SSc. The role of PTKs in the pathogenesis of fibrosis and SSc is well-established and PTK inhibitors have proceeded to clinical trials in SSc. There are very few reports on PTPs in SSc. However, the fundamental knowledge we have of PTP function in basic cell biology suggests that several PTPs might play important roles in regulating pathogenic pathways that underlie SSc development and severity. The majority of PTPs likely inhibit SSc pathogenic pathways and could be subjected to pharmacological activation for therapeutic purposes. Other PTPs such as SHP2, which promotes multiple signaling pathways, or VEPTP and RPTP α , which promote angiogenic and fibrogenic signaling, might become promising targets for pharmacological inhibitors. Table 2 summarizes the potential roles of key PTPs in SSc pathogenic pathways as described in the sections above.

After carrying for more than a decade a non-enviable reputation as “undruggable enzymes” due to well-known problems in development of orthosteric competitive inhibitors, PTPs are experiencing a resurgence as possible targets for allosteric inhibitors (see Fig. 2 for exemplified scheme of allosteric PTP inhibition) and perhaps in the future for pharmacological activators. A seminal report in 2016 described the discovery of an allosteric inhibitor of SHP2 (SHP099) which has unique features of potency, selectivity and bioavailability, and targets an allosteric pocket located between the SH2 domains and the catalytic domain of the PTP (116). These and other initial successes of allosteric targeting are leading to a renewed interest in drugging PTPs in academia and industry. Interestingly, at the dawn of PTK drug discovery, PTKs were also considered very difficult targets (117). However, continued drug discovery efforts eventually enabled the development of the PTK inhibitors that are now available for the therapy of cancer and other indications including fibrosis (118). The history of the closely related PTK field and the latest developments in the PTP field suggests that persistence might pay off in the PTP drug discovery field. Thus, expanding our knowledge of the role of PTPs in SSc is warranted, as it might pave the way to development of new classes of PTP inhibitors/activators to treat SSc and/or its complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The SSc pathogenic triad occurs through the interconnection between inflammation and autoimmunity, vascular injury and fibrosis. The major metabolic pathways in SSc pathogenesis are represented clustered around the triad components. Red arrows show inflammation and autoimmunity influence, and include the interleukin receptors 4 (IL-4R), 6 (IL-6R), 13 (IL-13R), toll-like receptor 4 (TLR4) and Fc gamma receptor (FcγR) signaling pathways. Blue arrows show vascular injury affecting SSc, including vascular endothelial growth factor receptor (VEGFR), prostacyclin receptor (PTGIR) and endothelin receptor (ETa-b) signaling pathway. Green arrows show fibrotic effects such as transforming growth factor beta (TGFβ), platelet-derived growth factor receptors (PDGFR), connective tissue growth factor (CTGF) and lysophosphatidic acid receptor 1 (LPAR1) signaling pathways. PTPs affecting those pathways are showed with a ↓ or ↑ arrow to indicate the inhibitory or enhancing effect.

Figure 2. PTP activity can be controlled allosterically using small-molecules to bind PTP-specific pockets, which in turn generate a conformational modification in the catalytic site.

Table 1.

Classification system of protein tyrosine phosphatases (PTPs). Class I includes the classical Tyr-specific transmembrane and non-transmembrane PTPs and different subclasses of dual-specificity PTPs (DSPs). Class II includes the low molecular weight PTP (LMPTP). Class III contains the Tyr/Thr-specific CDC25 PTPs.

PTP classification	
Class I	- Classical PTPs: 21 transmembrane (PTPR) and 17 non-transmembrane (PTPN) PTPs - Dual-specificity PTPs (DSPs): includes 11 Map Kinase Phosphatase (MKP) DSPs, 19 atypical DSPs, 5 Phosphatase and Tensin Homolog (PTEN) DSPs and others
Class II	- Low molecular weight PTP (LMPTP)
Class III	- 3 CDC25 PTPs

Table 2.

Protein tyrosine phosphatases (PTPs) involved in the regulation of SSc signaling pathways. Table shows the positive or negative effects on each SSc-associated pathway.

Gene	Protein/alternative names	Target pathways
<i>PTPRA</i>	RPTP α	TGF β $\uparrow$
<i>PTPRB</i>	RPTP β / VEPTP	VEGF $\downarrow\uparrow$
<i>PTPRC</i>	B220 / CD45	IL-6 $\uparrow$, TLR $\downarrow\uparrow$, Fc γ R $\downarrow$
<i>PTPRE</i>	RPTP ϵ	IL-6 $\downarrow$
<i>PTPRJ</i>	DEP1 / CD148	PDGF $\downarrow$
<i>PTPN1</i>	PTP1B	VEGF $\downarrow$, PDGF $\downarrow$, LPA $\downarrow$
<i>PTPN2</i>	TCPTP	IL-6 $\downarrow$, TGF β $\downarrow$, PDGF $\downarrow$
<i>PTPN4</i>	PTPMEG	Fc γ R $\downarrow$
<i>PTPN6</i>	SHP1 / PTP1C	IL-4 $\downarrow$, IL-13 $\downarrow$, IL-6 $\downarrow$, Fc γ R $\downarrow$, VEGF $\downarrow$, Prostacyclin $\downarrow$, Endothelin $\downarrow$, LPA $\downarrow$
<i>PTPN9</i>	PTPMEG2	VEGF $\downarrow$
<i>PTPN11</i>	SHP2 / PTP1D	IL-4 $\downarrow$, Fc γ R $\uparrow$, VEGF $\downarrow$, Endothelin $\downarrow$, TGF β $\downarrow\uparrow$, PDGF $\uparrow$, CTGF $\downarrow$
<i>PTPN12</i>	PTPPEST	IL-4 $\uparrow$, IL-13 $\uparrow$, TLR $\downarrow$
<i>PTPN13</i>	PTPBL / PTP1E / FAP1	IL-4 $\downarrow$, IL-13 $\downarrow$
<i>PTPN22</i>	LYP	IL-6 $\downarrow$, TLR $\downarrow\uparrow$
<i>PTEN</i>	PTEN / PTEN1 / TEP1	TGF β $\downarrow$
<i>ACPI</i>	LMPTP / HAAP	PDGF $\downarrow$, Fc γ R $\downarrow$, VEGF $\downarrow$