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The ubiquitin proteasome system as a potential therapeutic target for systemic sclerosis

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1. Abstract

The present review aims to summarize available knowledge on the role of the ubiquitin-proteasome system (UPS) in the pathogenesis of scleroderma and scleroderma-related disease mechanisms. This will provide the reader with a more mechanistic understanding of disease pathogenesis and help to identify putative novel targets within the UPS for potential therapeutic intervention. Due to the heterogenous manifestations of scleroderma, we will primarily focus on conserved mechanisms that are involved in the development of lung scleroderma phenotypes.

2. Pathogenesis of scleroderma

Scleroderma is a heterogenous autoimmune inflammatory disorder characterized by progressive fibrosis of the skin and internal organs. The American College of Rheumatology describes the three pathogenic hallmarks of scleroderma as fibroblast dysfunction, small vessel vasculopathy, and production of autoantibodies, leading to excess extracellular matrix deposition and fibrosis [1]. In the United States, scleroderma affects nearly 100,000 people, with an incidence estimated to be ~20/million people with a prevalence of ~240/million [2–4]. Clinically, the consequences of irreversible skin and organ fibrosis can be devastating leading to long term disability and increased mortality, with the greatest effect from interstitial lung disease and pulmonary hypertension [5–7]. Alterations in several molecular processes have been identified in the initiation and progression of scleroderma-related organ fibrosis, including activation of the innate immune system, autoimmune responses of the adaptive immune system, and excessive transforming growth factor β (TGFβ) signaling driving fibrotic tissue remodeling [8–10]. The pathophysiology of SSc-ILD is complex and incompletely understood, but shares the common features of organ fibrosis. The process of fibrosis is thought to follow four general phases: injury, activation of effector cells, synthesis of extracellular matrix (ECM) components, and ECM deposition with inadequate resorption [11]. In scleroderma, both endothelial and epithelial injury are histopathological characteristics of the disease [12,13]. While the inciting injury or antigens are incompletely understood, these insults are associated with the presence of inflammation, activation of the innate immune

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system, and activation of the adaptive immune system [9,14–16]. These events activate tissue fibroblasts, which are the primary effector cells that synthesize and deposit excessive ECM components [17,18], which eventually culminates in organ fibrosis. This paradigm is an oversimplification of the complex pathological changes in SSc-ILD, as infiltrating immune cells, epithelial cell differentiation, and impaired injury resolution are all additional mechanisms that contribute to the phenotype [18]. These pathogenic changes are dependent on a complex network of genetic, transcriptomic, and post-translational factors leading to the clinical phenotype of organ fibrosis. One important post-translational mechanism regulating protein levels is the ubiquitin-proteasome system (UPS), which selectively targets proteins for degradation [19,20]. This review will focus on the role of the UPS and protein degradation in scleroderma to evaluate this disposal system as a new therapeutic target for systemic sclerosis with a particular focus on the activation of the innate and adaptive immune systems, as well as the profibrotic response that is driven by fibroblasts.

3. The ubiquitin-proteasome system

The UPS is the main non-lysosomal protein degradation pathway in the cell. It degrades proteins that are covalently tagged with a polyubiquitin chain into small peptides which are then used for amino acid recycling but also for MHC class I antigen presentation [21,22]. Ubiquitination is carried out in a multi-step process involving E1-activating enzymes, E2-conjugating enzymes, and E3 Ligases. In humans there exist two E1 enzymes, about 30 E2s, and over 700 E3 ligases (Figure 1). The substrate ubiquitin acceptor site is typically a lysine, whereby the epsilon-amino group (ε-NH+3) forms an isopeptide bond with the carboxyl group (COO−) of the C-terminal glycine of ubiquitin. After a substrate is modified by a single ubiquitin moiety, it may be altered further by the transfer of additional ubiquitin moieties linked to one of seven lysine residues of ubiquitin, creating poly-ubiquitinated or multi-ubiquitinated linear or branched chains. The degree and type of ubiquitination serve different purposes [23]. The present understanding is that substrates that contain K48-linked polyubiquitin chains are primarily degraded by the 26S proteasome, while substrates linked with K63-polyubiquitin chains function to regulate such diverse cellular activities as kinase activation and protein trafficking [24]. K48-mediated protein ubiquitination and subsequent

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degradation by the proteasome is a critical regulatory mechanism controlling stability of proteins involved in inflammatory, metabolic, neurologic, hematologic, oncologic, and age-related diseases [20,25]. Ubiquitinated substrates are also subject to removal of the 8.5 kDa ubiquitin moiety catalyzed by one of several families of deubiquitination enzymes.

Proteins that are modified by K48-linked polyubiquitin chains are proteolytically cleaved by the 26S proteasome consisting of one or two 19S regulator complexes bound to the symmetric and barrel-shaped 20S catalytic core [26]. While the multimeric 19S regulators confers binding of ubiquitinated substrates, their deubiquitination and ATP-dependent unfolding of proteins, the 20S core contains three distinct active sites that proteolytically cleave the unfolded amino acid chain after tunneling into the proteolytic chambers [27,28]. These active sites come in two flavors, the standard subunits beta 1, beta 2 and beta 5, and the inducible immunoproteasome subunits beta1i (LMP2), beta2i (MECL-1), and beta5i (LMP7) forming the standard 20S (s20S) and the immunoproteasome (i20S), respectively [29]. Immunoproteasomes are constitutively present in immune cells but can be induced in every parenchymal cell type upon stimulation with Interferon (IFN) γ or tumor necrosis factor (TNF) α [30]. Although, s20S and i20S do not largely differ in their overall structure [31], they diverge in their cleavage site preference thus generating distinct sets of peptides upon substrate degradation. Immunoproteasomes thereby enable the more efficient generation of MHC class I ligands and have been shown to improve MHC I mediated immune responses [32]. In addition, accumulating evidence suggests a role for immunoproteasomes in the production of inflammatory cytokines, Th1 and Th17 differentiation, B cell maturation, autoimmune responses, and alveolar macrophage polarization [30,33]. The mechanisms whereby the immunoproteasome is mechanistically involved in these diverse processes are, however, still enigmatic.

4. Targeting the UPS in scleroderma

Targeting of the UPS in fibrotic diseases by small molecules is feasible on several levels: i) specifically interfering with defined E3 ligases, ii) inhibition of deubiquitinating enzymes, iii) catalytic inhibition of the proteasome or specific immunoproteasome active sites. The

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targeting of E3 ligases offers conceptual advantages when compared to, for example, the extensive array of compounds targeting protein kinases, some of which are in clinical use. Unlike protein kinases where several competitive inhibitors target structurally similar active sites that bind ATP, E3 ligases share distinct binding pockets or pharmacophores that do not depend on metabolite binding but are involved in substrate-ligase complex interactions [34]. As an example for an E3 ligase inhibitor, we would like to refer to the newly developed compound BC-1215, that exerts cytokine blocking activity by inhibiting the actions of the pro-inflammatory E3 ligase subunit, Fbxo3 [35]. Whether Fbxo3 inhibitors could reduce cytokine-driven inflammation in scleroderma will require further studies using relevant preclinical models [36]. Another compound, pevonedistat, that targets neddylation, a process required for activity of some ubiquitin E3 ligases, was shown to reduce scleroderma graft-versus host disease in a murine model [37]. Collectively, these observations provide a rationale for selectively targeting checkpoints in pro-fibrotic and pro-inflammatory pathways driven by E3 ligases in scleroderma.

While designing therapies to interfere with E3 ligase activity have distinct advantages, there are also known limitations. There are several hundred known E3 ligases that target thousands of cellular proteins. As each specific E3 ligase generally targets more than one substrate for degradation, there is potential for off target effects [38]. For example, the E3 ligase SCF^{FBXL2} has been shown to target several different substrates [39], so interfering with SCF^{FBXL2} activity might affect protein levels of all of its substrates. Additionally, the entirety of the E3-substrate interactome is only partially characterized, and the majority of E3-substrate interactions have not been characterized [40,41]. These factors present significant challenges in designing therapies aimed to affect a specific substrate protein by modulating activity of the E3 ligase that targets it.

In addition to E3 ligase inhibition, a variety of small molecule inhibitors have been developed serving as catalytic inhibitors of the proteasome. These compounds bind covalently or non-covalently to the catalytic sites of the 20S proteasome with different specificities thereby reversibly or irreversibly inhibiting its protease activities [42]. One prominent example for a reversible proteasome inhibitor is the first FDA-approved inhibitor Bortezomib (Velcade©) which has been successfully applied for the treatment of multiple myeloma patients since

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2003 [43]. However, reports on adverse systemic effects and reported nonspecific off-target effects limit the use of this compound. In addition, tumor cells develop resistance against catalytic proteasome inhibitors either by regulating proteasome levels in the cell or by acquired mutation of the active site [44–46]. Irreversible inhibitor binding induces sustained proteasome inhibition, as recovery of proteasome activity requires de novo synthesis of 20S core particles. However, these molecules exhibit a negative pharmacodynamic profile since they also inhibit proteasomes of healthy and non-malignant cells when administered intravenously [47]. All proteolytic subunits bind to the inhibitors via a common mechanism involving the nucleophilic addition of their Thr1 hydroxyl group to the inhibitor analogously to the nucleophilic attack of peptides for degradation [48]. Of note, the composition of side chains of the peptide scaffold of the inhibitor - but not the reactive group or the peptide backbone - defines the substrate specificity of the inhibitor. Besides covalent inhibitors, different classes of molecules interacting with the proteasome catalytic subunits in a non-covalent fashion have been generated, such as cyclic or noncyclic peptides [48]. Several inhibitors of specific catalytic subunits were developed, such as the β5-specific inhibitor oprozomib (ONX 0912). In addition, several immunoproteasome specific inhibitors have recently been developed which represents another milestone in proteasome inhibitor discovery (Figure 2): ONX 0914 or PR-924 which targets LMP7 [49], LMP2-specific inhibitors such as UK-101, LU-001i and KZR-504 [50–52], and the immunoproteasome-specific inhibitor LU-005i [53]. These inhibitors specifically inhibit either defined active sites of the immunoproteasome or act as pan-immunoproteasome inhibitors. They thus confer cell-specific activity as the immunoproteasome is constitutively expressed only in immune cells. The immunoproteasome constitutes generally about 50% of the overall proteasome content even in immune cells. Therefore, immunoproteasome inhibitors are generally well tolerated and show a comparatively large therapeutic window for treatment of diseases that involve unwanted activation of immunoproteasomes such as in autoimmunity [33]. A novel concept of proteasome inhibition involves the competitive or non-competitive inhibition of the binding of proteasome activators to the outer alpha rings of the 20S catalytic core as proposed by Gaczynska & Osmulski recently [54]. However, there are no drugs available yet to test this concept in systemic sclerosis.

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5. The UPS in scleroderma related innate immune receptor pathways

The innate immune system sits at the interface between the host and the environment, and it is the first line of defense against both invading pathogens or host-derived "danger" signals [55]. Pattern recognition receptors (PRR) on innate immune cells sense invading pathogen associated molecular pattern (PAMP) or host-derived damage associated molecular pattern (DAMP) molecules, causing activation of inflammatory signaling pathways and secretion of pro-inflammatory cytokines. In scleroderma, a number of ligands of both endogenous and microbial origin have been shown to promote the release of inflammatory mediators through binding to and activation of PRRs. Consequently, activation of the innate immune system and release of pro-inflammatory cytokines serves as a major stimulus for subsequent wound healing responses that underlie the fibrosis seen in the disease [9,16,56]. There is much interest in therapeutic approaches to disrupt these pathways, including targeting defined E3 ligases that regulate PRR's. Below we review PRR's implicated in scleroderma and the corresponding E3 ligases known to regulate them. Therapies designed to modulate these E3 ligases would aim to reduce aberrant innate immune activation and the subsequent inflammatory signaling that follows (Figure 3).

TLR2: Toll-like receptor 2 (TLR2) is a cell-surface pattern recognition receptor that classically recognizes peptidoglycan of gram-positive bacteria [57]. In addition, TLR2 is also activated by the endogenous acute phase reactant protein serum amyloid A (SAA), triggering TLR2-dependent inflammatory signaling and cytokine release [58,59]. Importantly, SAA is elevated in the sera of scleroderma patients [60] and may serve as an endogenous stimulus driving TLR2 activation. SAA increased IL-6 secretion in a TLR2-dependent manner in dermal fibroblasts [61], suggesting that the SAA/TLR2 signaling axis may be a contributor to the inflammatory component characteristic of scleroderma. TLR2 was further implicated as a contributor to inflammation in scleroderma in a large population study, where a rare genetic polymorphism in TLR2 (Pro631His) was associated with high levels of anti-topoisomerase antibodies in serum and development of pulmonary arterial hypertension in a large cohort of scleroderma patients [62]. In the same study, monocyte-derived dendritic cells from patients with the TLR2 Pro631His genotype secreted higher levels of proinflammatory cytokines TNFα and IL-6 [62], further suggesting a role for TLR2 signaling as contributor to scleroderma-related chronic

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inflammation. Thus, TLR2-driven processes may be a novel approach to limit inflammation in scleroderma. Our laboratory has recently identified the novel E3 Ligase PP1R11 as a negative regulator of TLR2 signaling [63]. PP1R11 is a member of the RING (Really Interesting New Gene) finger E3 ligases. PP1R11 ubiquitinates TLR2 and targets it for proteasomal degradation. *PP1R11* over-expression reduces TLR2-dependent cytokine production, while PP1R11 inhibition augmented TLR2 signaling. Hence, PP1R11 has not been examined in the context of TLR2-related signaling in scleroderma but may represent a novel target for future studies (Figure 3).

TLR4: Toll-Like Receptor 4 (TLR4) has also been implicated in the pathogenesis of scleroderma. TLR4 senses lipopolysaccharide (LPS) from gram-negative bacteria, but is also activated by several endogenous ligands, including extracellular matrix components up-regulated in scleroderma such as hyaluronic acid (HA), fibronectin extra Domain A, and Tenascin C [64,65]. Several studies have examined the role of TLR4 signaling in scleroderma. Bhattacharyya et. al showed that chronic activation of TLR4 signaling drives a pro-fibrotic phenotype by promoting collagen synthesis and inhibiting profibrotic responses [66], while Takahashi et al discovered that TLR4-/- mice are protected using a bleomycin-induced scleroderma mouse model [67]. Dendritic cells from scleroderma patients also secrete increased amounts of proinflammatory cytokines compared to healthy controls [68]. Taken together, these studies suggest that chronic TLR4 over-activation in scleroderma directly contributes to a pro-fibrotic phenotype by up-regulating ECM components and also by augmenting pro-fibrotic signaling through TGFβ. Hence, attenuation of TLR4 signaling may be a novel anti-inflammatory strategy in scleroderma. TLR4 is regulated by the UPS, the E3 ligase(s) targeting TLR4 may be novel future drug targets for investigation. TLR4 is ubiquitinated and targeted for degraded by the E3 ligase RNF216 [69]. *RNF216* over-expression reduces TLR4 levels and TLR4-dependent signaling, while *RNF21*6 knockdown abrogates TLR4-dependent effects, including cytokine secretion. However, the role of RNF216 in scleroderma requires additional investigation (Figure 3).

TLR9: Toll-like Receptor 9 (TLR9) is an intracellular TLR, located in endosomes. TLR9 is classically activated by bacterial CpG DNA, but also binds to and is activated by several endogenous ligands, including host mitochondrial DNA [70]. TLR9 expression is up-regulated in

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myofibroblasts from scleroderma skin biopsies; additionally, scleroderma dermal fibroblasts have increased TLR9 levels and increase pro-fibrotic genes expression in response to CpG DNA [71]. Additionally, Farina et. al also showed that TLR9 activation through Epstein-Barr Virus infection induces a pro-fibrotic phenotype in scleroderma fibroblasts [72]. Thus, over-activation of the TLR9 signaling axis may augment pro-fibrotic signaling pathways, contributing to the pathogenesis of scleroderma. Similar to TLR2 and TLR4, attenuation of TLR9 signaling would also represent a reasonable strategy to reduce inflammation and fibrosis in scleroderma. Interestingly, TLR9 is also targeted by the E3 ligase RNF216 for ubiquitination and proteasomal degradation [69]. Both TLR4 and TLR9 share a cytoplasmic TIR domain, and given that RNF216 ubiquitinates both proteins, TLR4 and TLR9 may share a common "degron" sequence, that could mediate recruitment of RNF216. Such molecular signatures within Toll receptors that mediate substrate-E3 ligase interaction could be a basis for design of small molecule activators that modulate the fibrotic process in scleroderma. Taken together, study of the E3 ligases regulating TLR signaling are active areas of investigation. Agents designed to modulate E3 ligase activity in TLR signaling thus might represent unique targets for therapeutic intervention.

In summary, TLR2, TLR4, and TLR9 are innate immune receptors implicated in scleroderma, and preclinical data indicate that increased expression and hyperactivation of these receptors contribute to both inflammation and fibrosis. The mechanisms responsible for TLR overexpression in scleroderma are unknown. One unexplored hypothesis is that TLR overexpression is the result of a reduction in their ubiquitination and degradation, causing increased protein levels and augmented signaling in response to ligands. Thus, examining the role of PP1R11 and RNF216, which target TLR2 and TLR4 and TLR9, respectively, are two new areas for future investigation in scleroderma.

6. The immunoproteasome in scleroderma

Beyond targeting single E3 ligases involved in PRR-mediated innate immune signaling, catalytic inhibition of the immunoproteasome might represent a unique therapeutic approach for systemic sclerosis. Emerging evidence suggests that immunoproteasomes are

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involved in shaping innate immune responses at different levels [30,73]. Secretion of pentraxin-3, a specific pattern recognition protein that is secreted by neutrophils and macrophages to opsonize pathogens and dying cells [74], was found to be reduced in LMP7 deficient macrophages and upon inhibition of LMP7 [75]. Of note, serum levels of pentraxin-3 were recently show to be elevated systemic sclerosis (SSc) patients compared to healthy controls and correlated with disease severity and ulcer formation [76]. Immunoproteasomes also directly affect macrophage function and may thereby modulate scleroderma pathogenesis: the absence of the immunoproteasome subunit LMP7 or specific inhibition of LMP7 in alveolar macrophages augmented IL-4 or IL-13-driven macrophage polarization towards an M2 phenotype [77]. Polarization of bone-marrow derived macrophages, however, was not affected by LMP7 deficiency [78]. The absence of immunoproteasome subunits LMP7 and MECL1 also had a pronounced impact on the transcriptome of dendritic cells and altered the maturation of DCs [79]. Taken together, these data indicate that immunoproteasomes shape the innate immune response of various cell types such as macrophages and dendritic cells. In addition, immunoproteasomes are known to regulate the production of pro-inflammatory cytokines such as IL-6, IFN-γ, TNF-α, GM-CSF, and IL-23 thereby affecting innate immune signaling [33,80]. Several of these cytokines are known to be involved in the pathogenesis of scleroderma and therapeutic strategies aiming at blocking of these pathways have been tested although with differing outcomes [36]. Inhibition of immunoproteasomes might represent a novel approach to target inflammatory cytokine signaling in SSc (Figure 3).

7. UPS in autoimmunity

The contribution of the proteasome and namely the immunoproteasome to T and B cell mediated autoimmune responses in scleroderma has not been investigated. The immunoproteasome plays a prominent role in the generation and presentation of MHC class I epitopes [81]. Mice lacking one, two or all immunoproteasome subunits show severe deficiency in MHC I surface expression and epitope generation [73,82]. Accordingly, immunoproteasomes play an important role in CD8 T cell mediated adaptive immune responses against infected cells and in autoimmunity [83,84]. Indeed, some evidence has suggested a role for the immunoproteasome in CD8 T cell mediated autoimmune responses

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such as in type 1 diabetes and multiple sclerosis [85,86]. Immunoproteasome deficient mice develop early-stage multiorgan autoimmunity including symptoms of type I diabetes following irradiation and bone marrow transplantation which is mediated by autoreactive CD8 T cells [87]. Accordingly, immunoproteasome subunits may protect the inflamed tissue against autoimmune CD8 T cell responses [84,88]. Indeed, rare mutations in the immunoproteasome PSMB8 gene have been identified in severe autoinflammatory disorders [89–93]. Moreover, recent studies have identified recessive mutations of immunoproteasome and proteasome genes resulting in altered proteolytic activity of the proteasome and sustained production of type 1 interferons in patients with the rare, genetic autoinflammatory CANDLE syndrome (Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature) [94]. Taken together these data suggest that the absence or mutation of immunoproteasome subunits contributes to the onset of autoinflammatory and autoimmune diseases. Intact immunoproteasome function may thus be required to protect from the development of autoimmune activation that might contribute to the pathobiology of SSc. On the contrary, aberrant immunoproteasome expression has been observed in several other human autoimmune disorders [95–98] and experimental models of autoimmunity [99,100] and appears to drive autoimmune pathogenesis [33]. It is assumed that uncontrolled immunoproteasome activation affects autoimmune reactions mainly at the level of Th1/Th2 and Treg/Th17 differentiation [33,84]. Two hallmark studies showed that the inhibition of the immunosubunit LMP7 or its deficiency suppresses the differentiation of pro-inflammatory Th1 and Th17 subsets but increased generation of anti-inflammatory regulatory T Tregs *in vitro* [49,101]. Moreover, several pro-inflammatory cytokines (IL-6, IFN-γ, TNF-α, GM-CSF, and IL-23) are suppressed by impaired immunoproteasome function. This makes the immunoproteasome a novel target for autoimmune disorders. Accordingly, specific immunoproteasome inhibitors have been successfully tested in various experimental models of autoimmunity as reviewed elsewhere [33,102]. To date, several site-specific immunoproteasome inhibitors have been developed[103], most notably the LMP7-specific inhibitor ONX-0914 (KZR-616) which will be tested in a Phase 1b/2, multi-center study in patients with Systemic Lupus Erythematosus or Lupus Nephritis for clinical safety and efficacy (https://clinicaltrials.gov).

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Proteasome and immunoproteasome function has also been shown important for B-cell mediated humoral responses [104,105]. Proteasome inhibition is particularly efficient in secretory cells such as plasma cells which has led to the approval of several proteasome inhibitors such as Velcade™, Kyprolis™ , and Ninlaro™ for the treatment of multiple myeloma (see here for examples of clinical trials with proteasome inhibitors). This observation led to the application of proteasome and specific immunoproteasome inhibitors in allograft rejection in humans and several experimental models, however, with mixed results [106–110]. It might be worth considering this therapeutic concept also for B-cell driven autoimmune responses including SSc as suggested previously for autoimmune diseases with renal manifestations [111].

8. UPS in fibrotic remodeling

The rationale for using small molecules and related chemical entities in fibrotic diseases is based on the inflammatory component and key signaling elements that promote the pro-fibrotic phenotype. A mechanistic centerpiece for the pathobiology of fibrosis as seen in scleroderma is activation of the TGFβ signaling network that is modulated by ubiquitin E3 ligases [8]. TGFβ activates intracellular signaling by binding to cell surface receptors TGFβR1 and TGFβR2, causing recruitment and activation of downstream intermediaries including the SMAD family of proteins [8]. Ultimately, the consequences of TGFβ signaling alter gene transcription to drive collagen synthesis, cross-linking, and the secretion of other extracellular matrix components [8]. Several E3 ligases have been shown to be critical regulators of TGFβ signaling, and indeed, expression of several E3 ligases, including Smurf1, Arkadia, Synoviolin, NEDD4, and Pellino1 are upregulated in various models of fibrosis, in fibroblasts, or tissues [112]. These E3 ligases may play a role by mediating disposal of key proteins that antagonize TGFβ signaling, that increase TGFβ production, or enhance matrix deposition. Specifically, there is evidence that the E3 ligase, Smurf2, is reduced in scleroderma fibroblasts, but increased after TGFβ stimulation, resulting in increased actions of Smad2/Smad3 that mediate TGFβ signaling leading to fibrosis [113]. Inhibitors of Smurf2 have been generated and provide an opportunity for testing in preclinical fibrosis models [114]. Another E3 ligase termed Fibrosing-inducing E3 Ligase 1 (FIEL1) has recently been shown to

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promote fibrosis downstream of TGFβ [115]. FIEL1 targets a key negative regulator of TGFβ signaling - protein inhibitor of activated STAT 4 (PIAS4) - for ubiquitination and degradation. PIAS4 reduces activity of SMAD3 through several mechanisms, attenuating pro-fibrotic pathways downstream of TGFβ [116,117]. FIEL1 ubiquitination and degradation of PIAS4 augmented TGFβ pro-fibrotic signaling *in vitro*, and *FIEL1* overexpression augmented bleomycin-induced lung fibrosis, while *FIEL1* silencing in ameliorated fibrosis in the same model. Thus, inhibition of FIEL1 might serve as a novel strategy to reduce TGFβ-driven fibrosis, which is a central feature of scleroderma. In this study a first-in-class small molecule inhibitor of FIEL1 was generated, termed BC-1485. BC-1485 disrupted the FIEL1–PIAS4 interaction, reduced PIAS4 ubiquitination, and increased PIAS4 protein levels. BC-1485 reduced TGFβ-dependent gene transcription *in vitro*, and reduced lung fibrosis in a bleomycin mouse model of injury *in vivo*. Thus, inhibiting the ubiquitination and degradation of PIAS4 by BC-1485 may serve as a strategy to reduce TGFβ-mediated fibrosis and may be relevant in scleroderma [115]. Additional proof-of-concept and preclinical studies, however, are needed to assess whether PIAS4 is a valid target in fibrotic disease and whether chemical inhibition of this target is effective in other complementary models of fibrosis (Figure 3).

Catalytic inhibition of the proteasome has also been shown to mediate anti-fibrotic effects in several experimental models of tissue fibrosis such as of the heart, liver, kidney, skin and lung [118–120]. Antifibrotic effects involve attenuation of profibrotic TGFβ signaling [118,120] (Figure 3). However, results are controversial and hampered by the adverse side-effects of ubiquitous proteasome inhibition [121–123]. We have recently shown that TGFβ induced myodifferentiation of lung fibroblasts depends on an increased assembly and activation of the 26S proteasome [124]. Moreover, levels of the 19S regulatory subunit Rpn6 were elevated in an experimental model of lung fibrosis and in idiopathic pulmonary fibrosis lungs. Silencing of Rpn6 impaired assembly of 26S proteasome complexes and counteracted TGFβ-mediated myodifferentiation suggesting that specific targeting of 26S proteasome assembly may thus represent a unique therapeutic approach to counteract the profibrotic effects of TGFβ. Interfering with the interaction of proteasome activators and the 20S catalytic counterpart thus emerges as a promising therapeutic strategy that might also be applied in the setting of SSc [54]. The involvement of the immunoproteasome in fibrotic tissue

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remodeling and in scleroderma-related lung fibrosis has not been investigated so far and remains to be unraveled.

9. Conclusion and outlook

Taken together, targeting the UPS in SSc represents a novel therapeutic approach which is, however, not well investigated. There are several lines of evidence that the use of specific inhibitors of E3 ligases may be useful to interfere with defined pathobiologic mechanisms in the course of SSc such as TLR signaling and profibrotic TGFβ signaling (Figure 3). In addition, the application of newly developed immunoproteasome inhibitors may be beneficial to counteract innate and autoimmune signaling thereby providing a more specific approach of targeting defined proteasome complexes in distinct cell types compared to the use of wide-spectrum proteasome inhibitors that are hampered by their adverse side effects (Figure 3).

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Figure legends

Figure 1: Protein degradation is mediated by the ubiquitin proteasome system. Ubiquitin is transferred to E1 activating enzymes in an ATP-dependent fashion, followed by ubiquitin transfer to E2 conjugating enzymes. E3 ligases recognize "degrons" on substrate proteins created by modifications such as phosphorylation. These ligases link substrate proteins to the ubiquitin-transferring machinery, and with further ubiquitination events (K48-linked poly-ubiquitin), substrates are shuttled to the proteasome for degradation.

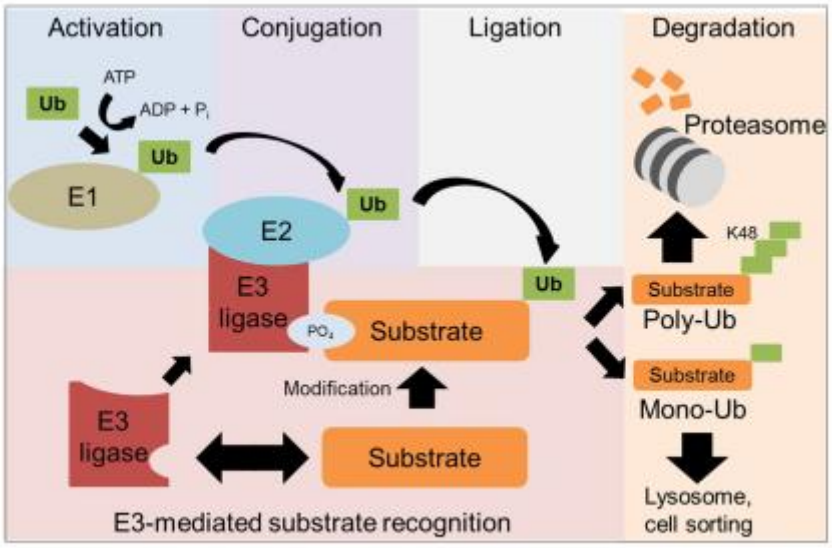

Figure 1

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Figure 2: Targeting of the immunoproteasome by site-specific immunoproteasome inhibitors reflecting the current state of drug development.

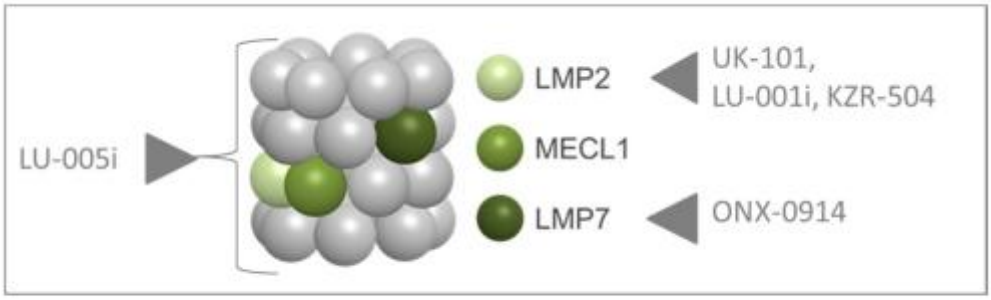

Figure 2

Figure 3: Targeting the UPS in systemic sclerosis. Diagram illustrating small molecule compounds against various targets that mediate innate immune function, autoimmunity, or the fibrotic response in scleroderma. These compounds could be potentially useful in this disorder but require further evaluation.

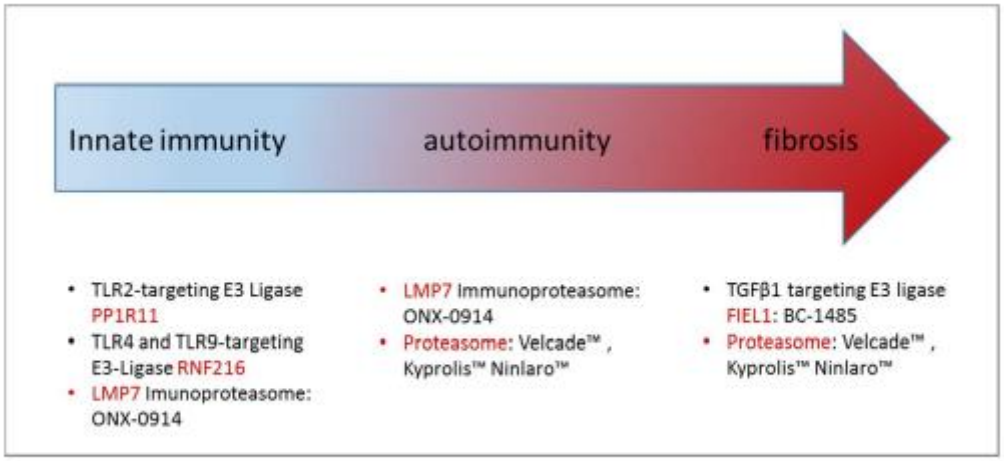

Figure 3