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Targeting STAT4 in systemic sclerosis: a promising new direction

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Abstract

STAT4 has been identified as a genetic risk factor for the development of autoimmune diseases including systemic sclerosis. STAT4 regulates Th1 cell development and cell-mediated immunity, but it is not known how it may regulate the development of dermal fibrosis. Using the bleomycin-induced dermal fibrosis model, it has now been demonstrated that STAT4-deficient mice have reduced dermal fibrosis in part via STAT4-dependent alterations in T-cell proliferation and cytokine production. These data stress the importance of STAT4 in autoimmune diseases such as systemic sclerosis and provide an important direction for future research to improve our understanding of systemic sclerosis pathogenesis.

Keywords

dermal fibrosis; STAT4; systemic sclerosis

Systemic sclerosis (SSc, scleroderma) is a multi-system disease clinically characterized by progressive fibrosis of the skin and internal organs [1]. Although the underlying pathogenesis is not fully understood, multiple lines of evidence point to inflammation and autoimmunity as a cause of the disease. Indeed, biopsies of early SSc skin demonstrate perivascular infiltrates of mononuclear inflammatory cells that lead to increased numbers of myofibroblasts and increased dermal thickness with thick collagen bundles found in biopsies of established SSc skin [2]. Autoimmunity is best exemplified by the presence of multiple but nonoverlapping SSc-associated autoantibodies that help define unique clinical subsets of patients [3]. Additional support for immune alterations in SSc comes from studies demonstrating increased circulating cytokines and, more recently, the identification of increased expression of genes involved in the type I interferon pathways [4,5]. More recently, genetic studies have strongly implicated autoimmunity and inflammation in SSc pathogenesis. These studies have demonstrated associations of polymorphisms in multiple genes involved in regulation of the immune responses such as *IRF5*, *TNFSF4*, *PTPN22* and *STAT4*. (reviewed in [6]).

STAT4 is a member of the signal transducer and activators of transcription (STAT) family of molecules. These proteins are the molecular link from the cell surface cytokine receptors to the nucleus, where they serve as critical transcription factors. STAT4 expression is relatively restricted, with high expression in lymphoid and myeloid tissue, although more

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recent studies suggest that it might be expressed in other tissues as well. STAT4 is phosphorylated in response to IL-12 and IL-23 receptor activation, and is an important regulator of Th1 development [7,8]. STAT4 is also activated in response to type I interferons. Polymorphisms in the STAT4 gene region have been associated with susceptibility to autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus [9]. More recently, STAT4 has also been identified as a susceptibility factor for the development of SSc [10].

How these polymorphisms in STAT4 alter its function and lead to the development of these autoimmune diseases remains unknown. Furthermore, a role for STAT4 in the development of SSc has not been determined either. The recent article published by Avouac and colleagues sought to determine the contribution of STAT4 to the development of dermal fibrosis in two murine models of dermal fibrosis [11].

Methods & results

In the current report, STAT4-deficient (*stat4*$^{-/-}$) mice were compared with wild-type mice in two different models of dermal fibrosis. The first model of fibrosis evaluated was bleomycin-induced dermal fibrosis. In this model, mice are administered daily subcutaneous injections of bleomycin or saline for 3 weeks starting at 6 weeks old. The TSK-1 murine model was also used. This mouse model is caused by a dominant mutation in the fibrillin 1 gene and is less dependent on inflammation. In both models, the amount of fibrosis in the skin was quantified and compared in *stat4*$^{-/-}$ mice and wild-type mice.

Using the bleomycin-induced dermal fibrosis model, the authors demonstrated that *stat4*$^{-/-}$ mice had reduced dermal thickness after bleomycin injection compared with wild-type mice. There was also a reduction in accumulation of collagen and the number of α-smooth muscle actin-positive myofibroblasts in the dermis of *stat4*$^{-/-}$ mice relative to wild-type mice. These data support an important role for STAT4 in the matrix deposition and the development of dermal fibrosis.

In addition to changes in fibrosis, *stat4*$^{-/-}$ mice also had a reduction in dermal inflammation after bleomycin injection. S*tat4*$^{-/-}$ mice had a reduction in infiltrating leukocytes, in particular $CD4^+$ and $CD8^+$ T cells, but not B cells or monocytes. The reduction in numbers of Ki-67^+ T cells suggests that STAT4 regulated T-cell proliferation in lesional skin. To further characterize the reduction in inflammation, cytokine levels in serum and skin lysates were compared. Subcutaneous bleomycin resulted in a relative increase in serum and dermal IFN-γ, IL-6 and IL-2 in wild-type but not *stat4*$^{-/-}$ mice. Interestingly, *stat4*$^{-/-}$ mice had similar levels of dermal IL-4, IL-5 and TGF-β after bleomycin injection compared with wild-type mice despite the reduction in dermal fibrosis. Together, these data suggest that STAT4 contributes to the development of dermal fibrosis in part by controlling T-cell proliferation and cytokine production.

In contrast to the bleomycin-induced fibrosis model, fibrosis in the skin in the TSK-1 model was not dependent on STAT4. S*tat4*$^{-/-}$ mice were crossed with the TSK-1 mouse to generate mice with the fibrillin 1 mutation and lacking *stat4* (*stat4*$^{-/-}$ /Tsk1). There was no difference in hypodermal thickness, extracellular matrix deposition, and numbers of myofibroblasts between *stat4*$^{-/-}$/Tsk1 mice versus wild-type controls. These data demonstrate that STAT4 is not required for dermal fibrosis in the TSK-1 mouse model.

Discussion

In the current report, Avouac and colleagues demonstrate a role for STAT4 in the development of dermal fibrosis. These data are the first to show a functional importance of

STAT4 for dermal fibrosis with important implications for SSc. Lesional skin from *stat4*$^{-/-}$ mice had reduced numbers of fibrotic and inflammatory end points in the bleomycin-induced skin fibrosis model, but not the TSK-1 model. While inflammation is classically seen in the bleomycin model, the TSK-1 model is characterized by fibroblast changes and less dependent on inflammation. The data suggest that STAT4 regulates dermal fibrosis through orchestration of leukocyte infiltration, proliferation and cytokine production. However, they do not rule out the possibility that STAT4 may regulate other cellular populations such as the fibroblast in other systems. Additional studies such as bone marrow chimeric experiments would be of great value in further substantiating this hypothesis and are important to advance our understanding of how STAT4 mediates dermal fibrosis and SSc.

STAT4 polymorphisms are associated with susceptibility to SSc [10,12,13]. A comparison of SSc patients and healthy controls in five European cohorts demonstrated an association of the *STAT4* rs7574865 T allele with SSc [10]. This association has been confirmed in multiple studies, including a recent genome-wide association study [12-15]. Gene–gene interaction studies have also demonstrated an additive effect of *STAT4* and *IRF5* with regards to SSc susceptibility [12]. Furthermore, an interaction of *STAT4* with *TBX21*, which encodes a transcription factor involved in Th1 development, has also been demonstrated with regards to SSc susceptibility [13]. Together, these studies identify and confirm that *STAT4* is an important genetic risk factor for the development of SSc.

The current paper sheds light on the mechanism by which STAT4 contributes to the development of dermal fibrosis. After administration of bleomycin, *stat4*$^{-/-}$ mice had reduced levels of Th1 cytokines, IFN-γ and IL-2, with similar levels of Th2 cytokines, relative to wild-type mice. It is known that STAT4 activation is a key step in the IL-12-induced differentiation of naive T-helper cells into Th1 cells [7]. There have been conflicting reports in the literature regarding the role of the Th1/Th2 cytokine balance in SSc, which may reflect different stages of the disease process or heterogeneity amongst SSc patients [16-18]. However, based on the genetic association of STAT4 with SSc, the importance of STAT4 in the Th1/Th2 cytokine balance and the current findings it is likely that STAT4 regulation of T-cell function plays a role in SSc pathogenesis.

In addition to Th1 development, STAT4 may also contribute to the development of dermal fibrosis through additional pathways. Genetic and functional studies have implicated type I interferons in SSc [5,19]. Type I interferons induce an inflammatory response in dermal fibroblasts that is accentuated in dermal fibroblasts from SSc patients relative to normal control subjects [20,21]. Interestingly, type I interferons (IFN-α and -β) can also promote STAT4 activation and induce IFN-γ production by Th1 cells. However, type I interferon activation of STAT4 has been observed in human but not murine T cells due to species-specific differences in the cytoplasmic domain of the IFNAR2 subunit [22]. Therefore, the murine models used to determine the molecular pathways involved in the development of dermal fibrosis are suboptimal to investigate the potential role of STAT4 in the type I interferon response and the current data cannot rule out these possibilities. It therefore remains possible that in SSc, STAT4 and its polymorphisms could lead to alterations in the type I interferon pathways in T cells or dermal fibroblasts.

In summary, multiple genetic and functional studies now implicate STAT4 as a mediator in the development of dermal fibrosis. Through the regulation of T-cell proliferation and cytokine production, STAT4 likely regulates fibroblast function and therefore dermal fibrosis. Future studies must determine the specific cytokine pathways and cells that are regulated by STAT4 and how these lead to the development of dermal fibrosis. In addition, it will be important to understand how the polymorphisms in STAT4 that are associated with

SSc susceptibility alter the expression and/or function of STAT4 and relate these changes to the pathogenesis of SSc. The paper by Avouac and colleagues provides us with the first functional studies of how STAT4 mediates dermal fibrosis, but is only the beginning.

Five-year view

Systemic sclerosis is a potentially devastating autoimmune disease with no proven disease-modifying treatments and minimally reliable diagnostic or prognostic biomarkers. STAT4 is emerging as a potential target that may translate to clinical care. It is not clear if STAT4 itself will be the target or if these data serve to identify potential pathways of autoimmunity and dysregulation. However, in the next few years, it is anticipated that our knowledge of how STAT4 regulates lymphocyte function and dermal fibrosis will expand. These data will allow us to better understand the pathways and network of proteins that lead to the development of SSc and help to identify cytokines and cellular targets. Indeed, the current paper noted that IL-6 levels were elevated in serum and skin of mice injected with bleomycin and reduced IL-6 in the absence of STAT4. These intriguing data suggest that IL-6 may then be a suitable therapeutic target in SSc.

Finally, over the next 5 years, our understanding of how polymorphisms in STAT4 alter immune function and lead to SSc will begin to emerge. The candidate gene association studies have provided valuable information about SSc pathogenesis, however, these studies are only the framework from which our understanding of this complex autoimmune immune disease will be built on. SSc is a polygenic autoimmune disease, and each polymorphism contributes a small, but important risk to its development. The key to understanding how STAT4 contributes to SSc pathogenesis will start with identifying the causal variants in the STAT4 gene regions and expanding our knowledge of gene–gene interactions. In the next few years, combining the genetic studies with human and murine functional studies will provide the foundation to translate these basic science findings to the clinical care of SSc patients.

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Key issues

- STAT-4-deficient mice demonstrated reduced fibrosis in the bleomycin-induced dermal fibrosis model.
- There was no difference in fibrosis in the STAT-4-deficient TSK-1 mouse model of dermal fibrosis compared to litter mate controls.
- STAT-4 may contribute to the development of dermal fibrosis through regulation of T-cell proliferation and cytokine production.