Molecular Mechanisms of Scleroderma and Fibrosis

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Abstract: Scleroderma is a chronic autoimmune inflammatory disease with widespread fibrosis of the skin and internal organs. Scleroderma is characterized by vascular injury, immune activation, inflammation and fibrosis. However, controversies related with the pathogenesis of scleroderma are still existing. Moreover, scleroderma is the most unsuccessfully treated disease in the rheumatic disorders. Despite prominent advances in the treatments of scleroderma related renal crisis, interstitial lung fibrosis and pulmonary arterial hypertension, treatment of skin involvement still continues to be a problem. The molecular mechanism of fibrosis should be clarified for the development of new drugs and treatment.

Keywords: Scleroderma, Fibrosis, Treatment

1. INTRODUCTION

Systemic sclerosis or scleroderma is a disease that shows involvement in internal organs or on the skin characterized by fibrosis. Dermis thickening and uncontrolled extracellular matrix (ECM) increase are seen in this disease whose pathogenesis is not fully understood (1,2).

Fibrosis may occur in all organs, but the first signs are usually seen on the skin. Fibrosis begins in the lower dermis with the involvement of subcutaneous fat tissue(2).There is not a certain treatment applied for Scleroderma yet.

2. SCLERO DERMA

Scleroderma is an autoimmune disease characterized with fibrosis (1-2). Clinically, systemic sclerosis is a heterogeneous disease with different sub-groups characterized by visceral involvement, measured by the presence of different antibodies (3,4).

It is a rare disease with a prevalence ranging from 50 to 300 in a million. As in many autoimmune diseases, women are under a higher risk than men (4-6).

Scleroderma is not a genetic disease (7). Many molecular pathways play an active role in this diseases and the relationship of all these pathways between each other is highly complex (8-15). Studies have shown that the disease plays a role in relation to extracellular matrix (ECM) genes and genes related to autoimmunity (control of natural immunity, functions such as the activation of macrophages and T-cells), as other immunological diseases.

The disease is most commonly seen at age 30-50 and the ratio of female to male is 8/1(16). In the United States, the incidence and prevalence of Scleroderma in the adult population were reported to be 19.3 and 242 in a million, respectively (5).

Although current treatment approaches control some findings of scleroderma, there is no internationally accepted treatment protocol for scleroderma (17). Scleroderma treatment should be regulated according to the organ systems involved and the severity of clinical findings. For this reason, when treatment is planned, the patients should be carefully evaluated in terms of organ system involvements. Currently, there is no approved basic effect drug that may be used in established fibrosis treatment (18). Corticosteroids are not effective on the disease progression(18). Additionally, corticosteroids may activate renal crisis and immunosuppressives such as azathioprine, chlorambucil and 5-fluorouracil have been found to be ineffective in scleroderma (19,20). The effect of D-penicillamine, which has been used for many years, is controversial (21). Target pathways and
molecular mechanisms are quite important in the development of new treatments with studies that have been made in recent years. Clarifying the molecular pathways of fibrosis and targeted treatments are quite important parts of scleroderma.

3. FIBROSIS MECHANISM IN SCLERODERMA

Fibrosis is the main mechanism in scleroderma. Fibrosis may occur in all organs but first signs are usually seen on the skin.) Fibrosis begins in the lower dermis with the involvement of subcutaneous fat tissue (2). The formation of fibrosis begins with the accumulation of active fibroblast cells and produced ECM structures (22). It is clear that excessive accumulation of ECM components with de novo synthesis is an important factor (23).

Thus, it contributes to the degradation and remodelling of ECM with fibrotic reaction (24). Figure 1 shows the environmental factors and pathogenesis that play a role in the development of fibrosis. More recently, it has been shown that macromolecule organization and component of ECM are altered by controlling tissue stiffness, growth factors and activation of cytokines (25-28).

![Figure 1: Fibrosis Pathogenesis](image)

Fibroblasts that go through many steps, by the activity of TGF-β, CTGF, PDGF and other factors, transform into (α-SMA) myofibroblasts characterized by α-smooth muscle actin (25,26). This process is completely a TGFβ- dependent process. By this time, many studies have been done on pathways related to fibroblast activation. Animal experiments have been done on scleroderma and studies are continuing for this pathway (29). It has been shown that in scleroderma fibroblasts, the mitogenic activity of PDGF receptors, a path way, is more sensitive. Also the activation of TGF-β receptors has been shown (38). The inferior pathways of TGF-β continue as Smad2/3 and Smad4. Smad 7, an endogenous inhibitor represses this pathway (25, 31). Some studies have shown that Wnt, Notch, Hedgehog, JAK-Stat pathways play an important role in fibrotic process (32, 33). With these findings, the role of Wnt β-katenin pathway in mesenchymal cells were supported with systematic analyses (34,35).In vitro experiments have shown the role of Wnt pathway’s collagen modelling, movement and reproduction of normal fibroblasts(34). Scleroderma is a disease characterized with immune system deficiency. Many mechanisms responsible from the immune system such as Th1, Th2 (auxiliary T cell responses) play a role (36-38). Figure 2 shows some cytokines related to systemic sclerosis and fibrosis, cellular sources of growth factors and biological activities (36-48).
### Table 1. Cytokines and growth factors functional in fibrosis (36-48)

<table>
<thead>
<tr>
<th>Cytokines and Growth factors</th>
<th>Cellular Source</th>
<th>Biological Activity</th>
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<tbody>
<tr>
<td>IFNγ</td>
<td>Lymphocytes, dendritic and NK cells (38,39,41,47)</td>
<td>Th1 differentiation, activation of B cells</td>
</tr>
<tr>
<td>TNFα</td>
<td>Macrophages, dermal mast cells and keratinocytes (36,37,38,44,46)</td>
<td>Neutrophil and lymphocyte healing, pro-inflammatory and pro-apoptotic response</td>
</tr>
<tr>
<td>Interlökin-1</td>
<td>Monocytes, macrophages, dendritic and endothelial cells (38,39,46)</td>
<td>Proinflammatory, interleukin-6 construction, PDGFβ Th1 and Th17 differentiation</td>
</tr>
<tr>
<td>Interlökin-2</td>
<td>T lymphocytes (38,44,46,47)</td>
<td>Stimulation of NK and CD8 + cells</td>
</tr>
<tr>
<td>Interlökin-5</td>
<td>Th2 and mast cells (36,37,38,41,47)</td>
<td>Differentiation of B cells</td>
</tr>
<tr>
<td>Interlökin-6</td>
<td>Fibroblasts, Th2 cells, macrophages, epithelial cells (38,39,41,42,43)</td>
<td>Collagen synthesis and inhibition of collagenase synthesis</td>
</tr>
<tr>
<td>Interlökin-10</td>
<td>Activated B cells and monocytes (38,40,41,43,44)</td>
<td>Collagen synthesis and immune response</td>
</tr>
<tr>
<td>Interlökin-13</td>
<td>Th2 cells, NK and Mast Cells (36,37,38,41)</td>
<td>B cell proliferation and differentiation, anti-inflammatory response and fibrosis</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Keratinocytes, Macrophages, Fibroblasts, T and B cells, platelets and endothelial cells (36,38,39,40,41,44,46,47,48)</td>
<td>Expression of fibroblast proliferation stimulation, expression of PDGF and TGFβ, production of CTGF and endothelin-1, Stimulation of collagen, fibronectin, proteoglycans, and TIMP (ECM destruction inhibitors) synthesis</td>
</tr>
<tr>
<td>CTGF (connective tissue growth factor)</td>
<td>Fibroblasts, Endothelial cells, smooth muscle cells (Induced by TGFβ, IL-4, and VEGF), (38,39,39,48)</td>
<td>Fibroblast proliferation, chemotaxis of fibroblasts in ECM production</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelets, macrophages, fibroblasts, endothelial cells (38,39,40,46,47,48)</td>
<td>Movement of fibroblasts, collagen, fibronectin and proteoglycan synthesis, TGFβ and IL-6 stimulation</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>Endothelial cells (38,39)</td>
<td>Fibroblast proliferation and collagen synthesis</td>
</tr>
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*+’marked molecules→Fibrosis-activating molecules, ‘X’marked molecules→Molecules involved in the inhibition of fibrosis, +, X’marked molecules→Molecules acts as both an activator and an inhibitor in the mechanism of fibrosis formation.

**Figure 3. Molecules involved in fibrosis**
4. COLLAGEN INCREASE AND BIOSYNTHESIS IN FIBROSIS

ECM and collagen increase is the most characterized feature of fibrosis which is the formation mechanism of scleroderma. Collagen proteins on the skin show a completely dispersed arrangement, forming a reticular structure. The covalent bonds between fibrils strengthen this structure. After being produced in the ribosomes of fibroblasts, collagen passes through endoplasmic reticulum and takes the helix form. Helix formation occurs as a result of prolin remaining from hydrolysis and hydroxyproline and hydroxylation of lysine.

Oxygen and ascorbic acid are needed for hydroxylation. This formation which takes the structure of helix is called procollagen (49, 50).

Procollagen exuded out of the cell from fibroblasts, is converted into a structure called tropocollagen by procollagen peptidase enzyme. Tropocollagen molecules synthesized inside the cell form the major components of collagen fibrils. Tropocollagen is a protein molecule whose molecular weight is about 300,000 daltons. It consists of 3 polypeptide chains of the same size that are made of 1000 amino acids. Collagen fibril ingenerates from 2 identical chains called α-1 and a third bond called α-2 coming together and forming a common helix structure. The collagen protein has 35% glycine and 11% alanine in its structure. Unlike other proteins, collagen contains 12% proline and 9% hydroxyproline. These amino acids are rarely found in other proteins. Glycine proline-hydroxyproline amino acids repeat continuously in the collagen structure.

Tropocollagen molecules come together in vivo conditions to form a left-handed Helix. In vitro conditions, it takes a quite long time for α-1 and α-2 bonds to form a triple Helix. Therefore, the percentage of this structure is lower when compared to total tropocollagen in the environment. (50). Collagen fibrils have long, crystal and filamentous structures that provide resistance to tissues (51). The fibrils are packed with connective tissue cells between them to provide support to the tissues. The bonds forming the collagen molecules contain an NH2 (α) and a COOH - (β) tip. The formation of mature collagen fibrils occurs when procollagen molecules, including α and β ends, are attached to each other. The procollagen molecules attached end to end have asymmetric tips (51). α tips are long and thin, β tips are shorter and thick. These fibril tips are first defined in the tendon tissue, then in the cornea and dermis (52, 53, 54). In scleroderma there is a direct proportion between the increase in collagen and the progression of the disease (55).

5. CONCLUSION

With the new studies, the molecular pathways in the fibrosis process of Scleroderma are becoming more prominent. It may be effective in treatment or in preventing fibrosis when a specific inhibition mechanism is revealed in ECM synthesis or in the molecular mechanism of sclerotic lesion formation (38).

In the clinic, there is a need to develop new strategies and therapeutic agents for the treatment of scleroderma.

REFERENCES


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