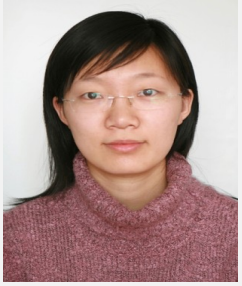
ImmunoTools *special* Award 2022



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Correlation of the high-throughput proteomic analysis with functional principles and disease manifestation in systemic sclerosis

Background

Systemic sclerosis (SSc) is a multisystemic autoimmune disease, which is characterized by its pathophysiological triad of microvascular damage, dysregulation of the immune system and a generalized fibrosis involving the skin and internal organs. SSc is categorized into a limited and a diffuse form, according to their skin involvement.

Furthermore, diffuse cutaneous systemic sclerosis (dcSSc) is characterized by a rapid disease progression and development of early organ manifestations, which determine the patient's clinical outcome.

The cause of SSc remains largely unknown, however understanding the key signaling pathway is crucial for the treatment of the disease. Nuclear factor-kappa B (NF-κB) is a transcription factor which is critical in the regulation of immunity and inflammation. It mediates the expression of inflammatory proteins including adhesion molecules, cytokines, growth factors and ECM metabolic regulators. The NF-κB transcription factor family is composed of five members including RelA, RelB, c-Rel, NF-κB1 and NF-κB2. It has been reported that deregulation of intracellular signal transduction by NF-κB takes place at the beginning of SSc and in its fibrosis stage.

A few cytokines are potent pro-inflammatory inductors of the NF-κB signaling pathway. Serum levels of Tumor necrosis factor α (TNFα) are elevated in SSc patients and are correlated with clinical features, such as pulmonary fibrosis. The expression of Interleukin-1β (IL-1β) in fibrotic skin tissue of SSc is found to be significantly up-regulated. Interleukin 17 (IL-17) has the ability to synergize with other cytokines like TNFα and TGF-β to promote and prolong inflammatory and fibrotic processes.

Objectives

Based on proteomics data and pathway analysis we found the conformationally changed peptides in NF-κB-related pathways. In this project, we aim to investigate the functional analyses of NF-κB signaling pathway in human dermal fibroblasts derived from dcSSc patients compared to dermal fibroblasts from healthy donors.

Project description

As a first step, we obtained dermal fibroblasts from dcSSc patients and healthy donors. We used Limited Proteolysis-coupled Mass-Spectrometry (Lip-MS) to examine protein structural alteration on a proteome-wide scale. LiP-MS was recently developed at ETH Zurich[1]. The two-step proteolysis allows us to compare proteomes subjected to different conditions. Differences in relative peptide abundance between the compared conditions indirectly implicate proteins that underwent structural changes. LiP-MS analysis detected a total of 53263 peptides in SSc skin fibroblasts, of which 41 peptides showed conformational changes in SSc fibroblasts in comparison with fibroblasts from healthy donors. SAE1, CTNND1, CDC37, and PPP1R13L were connected to the NF-κB pathway.

Based on these data, we will perform qPCR to evaluate the mRNAs in dermal fibroblasts samples. For stimulation, the starvation medium was supplemented with either TNFα, IL-1β, TGF-β, IL-17A, or a combination of IL-17A and TGF-β. The transcriptional activity of NF-κB family member and NF-κB pathway-related genes will be analysed, including RelA, NFKBIA, Interstitial Collagenase, Tenascin C, and Fibronectin 1.

Finally, we would like to perform the NF-κB activity measurement in different stimulation conditions by using NF-κB reporter assay. To examine the NF-κB activity in human dermal fibroblast, cells were transfected with a pseudotyped HIV-1-based lentiviral vector. The tdTomato sequence allows for identifying transduced cells by fluorescence microscope in addition to the real-time assessment of the NF-κB activity levels by the luciferase reporter expression.

Significance of the project

Taking into account novelty of our proteomics-based protein conformation data, we believe to propose entirely novel concept of signalling pathway regulation in fibrogenesis in SSc, especially the NF-κB signaling pathway. SSc is a rare disease, but its economic burden from a social perspective is substantial and outweigh the burden from other much more frequent diseases.

We assume that our findings will serve as a basis for developing new diagnostic tools and further for personalized, precise, and effective therapies tailored to the individual patient, decreasing the economic burden and improving the quality of daily life.

During this project the cytokines from ImmunoTools will be used in all in vitro experiments. In addition, ELISA kits will allow evaluating the cell functions.

References

[1] de Souza N, Picotti P. Mass spectrometry analysis of the structural proteome. Curr Opin Struct Biol. 2020;60:57-65.