ImmunoTools *special* Award 2016

Michal Rudnik, PhD-student

Supervisor: PD Dr Gabriela Kania

Division of Rheumatology, University Hospital Zurich

Monocyte/neurotrophins/organ fibrosis - novel axis in systemic sclerosis

Background

Systemic sclerosis (SSc) is a complex autoimmune disease characterized by microvascular damage, dysregulation of innate and adaptive immunity and general fibrosis in multiple organs such as skin, lungs and heart. The exact cause of SSc remains elusive, however understanding of key pathological pathways, cell types and mediators is crucial for development-targeted therapies. In other fibrotic diseases, bone marrow originated cells were indicated as a one of major sources of pathological myofibroblasts. These cells are not only capable of extracellular matrix production, but also affecting other cell types by secretion of many different cytokines and growth factors. Neurotrophins (NTs) are a family of proteins promoting neurons growth and survival and include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4). Profibrotic and proangiogenic actions of these factors were recently discovered in fibroblasts and endothelial cells. In addition, dysregulated serum levels of NTs were already shown in SSc patients. Neurotrophins interact with the Trk receptor family and the $p75^{NTR}$ receptor. Trk receptors bind with high affinity specific neurotrophins, while $p\text{-}75^{NTR}$ represents a low affinity receptor, to which all neurotrophins bind.

Objectives

In this project, we would like to determine the role of circulating monocytes in the onset and progression of fibrosis in SSc. First, we will examine monocyte-to-myofibroblast differentiation pathway(s) in SSc. Further, we will investigate the influence of neurotrophins on different cell types involved in the pathogenesis of SSc.

Project description

As a first step, we will screen skin, lung and endomyocardial biopsies from SSc patients and healthy controls for presence of bone marrow originated cells co-localizing with fibrotic markers, neurotrophins and neurotrophins' receptors.

Next, $CD14^{+}$ monocytes will be isolated from the peripheral blood of SSc patients and healthy donors and in vitro differentiated towards the myofibroblast phenotype by stimulation with profibrotic Immunotools cytokines, such as TGF-β1, IL-4, IL-10 and

IL-13. Expression level of neurotrophins (intracellular and secreted) and neurotrophins' receptors as well as profibrotic markers will be evaluated. In addition, we will check the effect of neurotrophins on dermal skin fibroblast originated from SSc patients, cardiac fibroblast from the iDCM patient and healthy subjects. In parallel, we will apply another approach mimicking different cell-to-cell interaction types. Dermal and cardiac fibroblasts will be directly and indirectly co-cultured with $CD14^+$ monocytes. This particular setting will allow us to investigate monocytes differentiation as well as activation of neurotrophins' signalling pathways in both cell types.

In our research, we would like to trace the fate of SSc and healthy control $CD14^+$ monocytes injected into NOD/SCID mice treated with angiotensin II, which leads to development of myocardial and dermal inflammation and fibrosis.

Last but not least, we plan to employ several mouse models, which mimic fibrosis in SSc such as: bleomycin induced skin and lung fibrosis, angiotensin II induced heart fibrosis and Fra-2 transgenic mice. We want to observe the expression of neurotrophins and neurotrophins' receptors during development of fibrotic multiorgan lesions. Further, we plan to block Trk/$p75^{NTR}$ signalling pathway and observe the effect on the development and progression of the multiorgan fibrosis. In that purpose, we will use chemical small molecule inhibitors of neurotrophins receptors as well as TrkA/B/C-Fc chimeric proteins, which specifically bind and block biological activity of neurotrophins.

Significance of the project

We expect to identify key cellular sources and molecular mechanisms of fibrogenesis in SSc. If we are able to successfully target these pathways in SSc animal models, this will be the basis for the development of novel and effective therapies targeting the often fatal involvement of internal organs in SSc patients.

During the project cytokines from ImmunoTools will be used in all cell stimulation experiments. Additionally ELISA kits and flow cytometry antibodies allow evaluating the cell phenotype.

ImmunoTools *special* AWARD for **Michal Rudnik** includes 25 reagents

FITC - conjugated anti-human CD66b, CD47, Annexin V

PE - conjugated anti-human IL-6, CD10, isotype control IgG2b

APC - conjugated anti-human CD29

human ELISA-set: human IFN-gamma, human IL-10, human IL-12p40 total

recombinant human cytokines: rh BDNF, rh GM-CSF, rh IL-4, rh IL-10, rh IL-13, rh IP-10 /CXCL10, rh beta NGF DETAILS more AWARDS