Abstract

Signaling pathways must be finely tuned to assign a signal of appropriate strength and duration to the receptor stimulation. Their dysregulation can be very harmful. The consequences of dysregulated signaling pathways vary from autoimmunity, immunodeficiency, and autoinflammation to abnormal proliferation and cancer. In my thesis I aimed to characterize the roles of kinases and membrane associated or transmembrane adaptor proteins in signaling pathways downstream of different receptors.

First, I was comparing the roles of SRC family kinases (SFK) in the initiation of antigen receptor signaling in B cells and in T cells. This effort resulted in the manuscript where we re-evaluated current data, which suggested that SYK can initiate BCR signaling independently of SFK. We show that much lower SFK activity is required for the initiation of BCR signaling than for TCR signaling, but we did not find any evidence for SFK-independent signal transduction. We also found that multiple factors are responsible for setting the higher threshold for SFK activity required to initiate signaling by TCR, including differences between SYK and ZAP-70, structure of the antigen receptor itself and separation of the receptor from transmembrane adaptor LAT, which is a major hub coordinating the formation of TCR signalosome. Based on our data, we propose that TCR detects the SFK activity at multiple levels. We further discuss how multiple level SFK kinase sensing model fits to the current model of TCR signaling and antigen discrimination.

Chemokine receptor CXCR4 is another key leukocyte receptor. It regulates homing and retention of leukocytes and their progenitors in the bone marrow and is essential for proper hematopoiesis. We found that it is negatively regulated by a novel previously uncharacterized transmembrane adaptor protein WW binding protein 1 like (WBP1L) also named Outcome predictor of acute leukemia 1 (OPAL1). WBP1L expression was reported to be upregulated in the most common type of childhood acute lymphoblastic leukemia but its function has been unknown. In the work presented in this thesis, we show that via the recruitment of NEDD4-family ubiquitin ligases WBP1L regulates the expression and activity of CXCR4 and very likely other CXCR4-independent pathways. This way it contributes to the control of migration and other functions of hematopoietic stem cells and hematopoietic progenitors, and to their ability to reconstitute hematopoiesis after bone marrow transplantation. Collectively, our data show that WBP1L

regulates hematopoietic processes, which are clinically relevant and may also have consequences for the biology of leukemia.

I have also contributed to the work aiming to characterize the mechanism of how another membrane-associated adaptor protein PSTPIP2 regulates inflammation. Absence of PSTPIP2 in mice leads to the development of autoinflammatory disease chronic multifocal osteomyelitis (CMO). It appears to be caused by dysregulated production of proinflammatory cytokine IL-1β by neutrophils. We found that reactive oxygen species (ROS) production by NADPH oxidase is also severely deregulated in neutrophils from these animals and that blocking NADPH oxidase by genetic deletion of its gp91phox subunit almost completely prevents the bone damage accompanying this disease. These data suggested that dysregulated ROS production is among the major pathophysiological mechanisms behind the autoinflammatory disease in PSTPIP2-deficient mice and may also be relevant for autoinflammatory bone diseases in humans.

Finally, for parts of these projects it was important to have a reliable method for expression of various cDNA constructs in hematopoietic progenitors and in dendritic cells and macrophages differentiated from these cells. The reliable cost-effective protocol we developed for this purpose was published separately and it is also a part of this thesis.

Collectively, the data presented in this thesis demonstrate crucial role of membrane adaptor proteins and kinases in the regulation of leukocyte signal transduction through various receptors. Consequently, they also demonstrate the fact that these proteins influence leukocytes at multiple levels, including their development, migration, and participation in the immune response.