ABSTRACT (EN)

This thesis is focused on two important gate-keepers of mast cell signaling. The first is the complex of the high-affinity receptor for immunoglobulin E (IgE) (FcεRI) associated with Lck/Yes-related novel tyrosine kinase (Lyn), which is involved in acquired immune responses and the second is the stromal interaction molecule (STIM)1, which senses calcium levels in endoplasmic reticulum (ER) and upon depletion of ER Ca^{2+} stores participates in opening of the plasma membrane Ca^{2+} release-activated Ca^{2+} (CRAC) channels.

Although the structure of FcεRI is known for many years and numerous molecules associated with the receptor have been described, the exact molecular mechanism of initiation and termination of the FcεRI signaling is elusive. Therefore, we evaluated the current knowledge on the molecular mechanisms of FcεRI phosphorylation with emphasis on the newly described model according to which cross-talk between protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs) sets the threshold for FcεRI tyrosine phosphorylation (PTK-PTP interplay model). Furthermore, we extended the knowledge about topography of active phosphatases which are prone to oxidation within the clusters of transmembrane adaptor proteins non-T cell activation linker (NTAL) and linker for activation of T cells (LAT) upon FcεRI triggering.

Using bone marrow-derived mast cells BMMCs as a model, we obtained new data on colocalization of STIM1 with microtubule filaments and movement of STIM1 in microtubule-dependent manner that reflected direct communication between STIM1 and microtubule plus-end tracking protein EB1. To determine whether STIM1 regulates EB1 movement and microtubules organization in calcium dependent manner we prepared BMMCs with reduced expression of STIM1. We found, as expected, that STIM1-deficient cells exhibited impaired calcium signaling upon activation and that this resulted in inhibition of de-novo reorganization of microtubules. Unexpectedly, treatment of BMMCs with the inhibitor of microtubules polymerization (nocodazole) failed to impaire translocation of STIM1 to ER/plasma membrane junctions and CRAC channels function. The cross-talk between microtubules and STIM1 was analyzed in detail by recording changes in reorganization of microtubules in BMMCs attached on fibronectin-coated slides after their activation with different stimuli (antigen, thapsigargin or pervanadate). BMMCs activated by all activators employed showed formation of distinct microtubule protrusions. This hitherto unknown mast cell function required co-stimulatory signaling from integrins. STIM1 was localized in the plasma membrane protrusions suggesting that local calcium signaling plays a role in formation of such protrusions.

Significant effort was also directed towards production of new monoclonal and polyclonal antibodies toward STIM1 and development of new real-time polymerase chain reaction (PCR) master mixes suitable for amplification of DNA fragments from whole blood and/or GC-rich templates. Outputs from these projects are either commercially available products (anti-STIM1 monoclonal antibody) or are in the processing for commercial use [new quantitative PCR (qPCR) master mixes].