ABSTRACT (EN)

This thesis focuses mainly on understanding of the regulatory roles of the transmembrane adaptor proteins, non-T cell activation linker (NTAL) and phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), in murine mast cell signaling. There are conflicting reports on the role of NTAL in the high affinity immunoglobulin E receptor (FcεRI) activation pathways in mast cells. Studies carried out on mast cells prepared from NTAL knock-out mice have indicated that NTAL is a negative regulator of FcεRI signaling, whereas experiments performed on human mast cells and rat basophilic leukemia cells with silenced NTAL expression have suggested its positive regulatory role. To thoroughly examine the involvement of NTAL in FcεRI-mediated signaling events in mouse mast cells and to determine whether different methodologies of NTAL ablation have different physiological consequences, we utilized a broad range of assays. Using bone marrow-derived mast cells (BMMCs) as a model, we obtained cells from NTAL wild type and knock-out cells and using lentiviral delivery approach we transduced part of the wild type cells, with vector bearing NTAL shRNA or empty vector to generate NTAL knock-down cells and control cells, respectively. Comparison of all four groups of generated cells in our assays revealed that both types of NTAL-deficient BMMCs exhibited enhanced degranulation, calcium mobilization, chemotaxis, tyrosine phosphorylation of linker for activation of T cells (LAT) and ERK and depolymerization of filamentous actin. These data provide evidence that NTAL is a negative regulator of FcεRI activation events in murine BMMCs, independently of possible compensatory developmental alterations.

To gain further insight into the downstream signaling activity of NTAL, we examined the resting and antigen-activated transcriptome profiles of all four types of generated BMMCs. Through this analysis we identified several genes that were differentially regulated in nonactivated and antigen-activated control and NTAL-deficient cells. Interestingly, a subset of these genes was involved in regulation of cholesterol-dependent events in antigen-mediated chemotaxis. The combined data indicate multiple regulatory roles of NTAL in gene expression and mast cell physiology.

We have also shown that another transmembrane adaptor protein, PAG, has both positive and negative role in mast cell FcεRI-mediated activation depending on the signaling pathway involved.

Since both studies demanded numerous quantitative real-time PCR examinations, part of the focus was also dedicated to development of new PCR master mixes suitable for amplification of difficult-to-amplify DNA fragments. We found excellent performance of a PCR mix supplemented with 1 M 1,2-propanediol and 0.2 M trehalose. This master mix is now also commercially available.

Lastly, we have reviewed recent approaches towards inhibiting mast cell mediated events in diseases or with mast cell related pathology, with the main focus being on recently developed inhibitors of intracellular signaling pathways and their relevance to clinical trials.