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Dissertations-Kurzfassung

Biochemical and functional characterization of stabilin-1 interacting synaptotagmin-like protein SI-SYT

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Synaptotagmins are a large family of proteins, which have been shown to be involved in diverse vesicular trafficking processes. The basic function of synaptotagmins lies in their ability to bring lipid membranes into close proximity by associating with cellular membranes and other transmembrane proteins like SNAREs. It has been shown that these molecular events can drive vesicle-vesicle or vesicle-plasma membrane fusions. SI-SYT (KIAA1228, FAM62B) is a novel synaptotagmin-like protein with unknown function, and it was discovered to interact with stabilin-1 in yeast two hybrid screening. Stabilin-1 is a scavenger receptor specifically expressed in alternatively activated macrophages and sinusoidal endothelial cells. Several functions including endocytosis and sorting of specific ligands (acLDL, SPARC, PL) have been ascribed for stabilin-1. Macrophages are highly versatile cells in the body which regulate many biological processes including inflammation, tissue homeostasis and remodelling as well as wound healing. So far only synaptotagmin 7 has been shown to participate in macrophage specific processes. Therefore, there is a vast gap of knowledge about the role of synaptotagmins in macrophage functions. The aim of this study was to characterize SI-SYT protein and to investigate its function in macrophages. At first the expression of SI-SYT was analysed by RT-PCR, which revealed that SI-SYT is at different levels expressed in various cell types (monocytes, T-cells, B-cells and tumour cells). Further it was shown that the expression of SI-SYT mRNA was increased 3-4 fold in human macrophages after stimulation with dexamethasone alone or in combination with IL-4. To analyse the expression profile and the intracellular localization of endogenous SI-SYT protein, specific rat monoclonal antibodies and rabbit polyclonal antibody were generated. Using these antibodies it was shown by WB that SI-SYT protein can be expressed in three different forms (full-length 95 kDa, approximately 68 and 64 kDa). In monocyte-derived cell lines, T- and B-cell line, the full-length protein was primarily expressed. In blood-derived macrophages, the expression of the smallest form was especially pronounced in M_{dex} and $M_{IL\text{-}4/dex}$, whereas in nonstimulated macrophages or in $M_{IFN\gamma}$, $M_{IL\text{-}4}$ and $M_{IFN\gamma/dex}$ the full-length SI-SYT was preferentially expressed. Analysis of SI-SYT intracellular localization by IF revealed SI-SYT protein being both cytoplasmic as well as at the plasma membrane. In human macrophages the localization of SI-SYT was changed upon different stimuli. In $M_{IFN\gamma/dex}$ SI-SYT was relocating to the plasma membrane whereas dexamethasone increased the localization at the TGN. Next, the colocalization of SI-SYT with different intracellular markers was analysed by IF. It revealed that SI-SYT is often localizing to cytoskeleton and occasionally to different vesicular markers, like TGN and clathrin. SI-SYT was colocalizing with stabilin-1 at the TGN and at the plasma membrane. Biotinylation assay confirmed the surface expression of SI-SYT in macrophages. The major part of SI-SYT positive vesicles were found to be localised to the ER. To investigate with which proteins SI-SYT interacts, yeast two hybrid screening was carried out with brain cDNA library and the full cytosolic domain of SI-SYT. Following proteins were discovered to interact with SI-SYT: SORBS2, KIAA1345, CATSPER2, MAP1B, KIAA1755, ATP1B, GFAP, ANLN, KIF5A, TTYH1, YIF1A, UNC119, MRPS26, FADS3, PPP1R9A, TAF1, EIF1B, MAPKAPK5, ZMYM5. Several of these proteins have been shown to associate with cytoskeleton, and some of them with vesicles. Kinesin heavy chain was selected for further analysis, because this protein has been shown to be the key molecule in the transport of vesicles along microtubules. SI-SYT was binding to neuronal kinesin heavy chain (KIF5A) via its C2A domain in yeast cotransformation. SI-SYT was also interacting with ubiquitous kinesin heavy chain (KIF5B) *in vivo* as shown by IP in HeLa and Jurkat cells and *in vitro* as shown by GST pull-down. In addition, KIF5B was expressed in human macrophages, where it occasionally colocalized with SI-SYT. The GST pull-down assay with different fragments of SI-SYT revealed the complexity of the interaction with KIF5B. There were either one or several binding sites in the C2B+C2C region, or an inhibitory site in

the C2A domain of SI-SYT protein. Several functional assays were performed in human blood derived macrophages. Inhibition of TGN-lysosomal trafficking by brefeldin A did not affect the localization of SI-SYT, but sustained its colocalization with TGN compartment. Stimulation of $M_{IFN\gamma/dex}$ but not the $M_{IL\text{-}4/dex}$ with LPS for 1h led to the decrease of SI-SYT at the plasma membrane and occasionally to the accumulation close to the TGN compartment. During placental lactogen endocytosis SI-SYT relocalized in leupeptin pre-treated macrophages close to the TGN at the area of PL storage vesicles. This effect was most pronounced after 1h of PL uptake. Also, leupeptin treatment led to the increase in expression of SISYT full-length protein (analysed by WB), especially after 1h and 3h of PL uptake. The data presented in the current study indicate that SI-SYT can have specific functions in human macrophages, in particular in the change of lysosomal enzyme tafficking upon bacterial stimulation and in facilitation of the transcytosis of hormone placental lactogen. SI-SYT could also regulate the trafficking between the ER and TGN, or the internalization processes at the plasma membrane.