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Dissertations-Kurzfassung

Cellular and molecular characterization of novel stabilin-1 interacting chitinase-like protein (SI-CLP)

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Macrophages are one of the most versatile cell types in the body, participating in a vast array of biological processes including fighting infections, tissue homeostasis and remodelling as well as wound healing. Alternatively activated macrophages actively orchestrate anti-inflammatory and healing processes. Stabilin-1 is a type 1 transmembrane endocytic/sorting receptor and is a marker for alternatively activated macrophages. Intracellular sorting function of stabilin-1 needs its interaction with GGAs (Golgi-localized, gamma-ear-containing, adenosine 5´-diphosphate-ribosylation factor-binding adaptor). Yeast-two hybrid screening resulted in identification of novel chitinase-like protein as an interacting partner of stabilin-1. SI-CLP (stabilin-1-interacting chitinase-like protein) contains a conservative Glyco_18 domain and belongs to the group of mammalian chitinase-like proteins. The members of this group include chitotriosidase, AMCase, YKL-40, YKL-39 and Ym1/Ym2, which possess cytokine activity and are associated with tumour progression, Th2 inflammations and allergies.

In the present work the regulation of expression of SI-CLP in human macrophages was analyzed using real-time RT-PCR and Western blotting. The expression of SI-CLP in macrophages was strongly up-regulated in combination of Th2 cytokine IL-4 and dexamethasone and this effect was suppressed by Th1 cytokine IFNγ but not affected by IL-10. SI-CLP expression was not restricted to primary monocyte-derived macrophages, and was also found to be high in MonoMac6, THP-1, Raji-cells and 293-HEK cells. Low level of SI-CLP protein was also detected in Jurkat, MCF-7 and HeLa cells, but not in H1299 cells.

Intracellular distribution of SI-CLP was analyzed by immunofluorescence/confocal microscopy using rat monoclonal anti-SI-CLP antibody (1C11). SI-CLP was found to be sorted to the late endosomes and secretory CD63–positive lysosomes in human alternatively activated macrophages, MonoMac6 and HUVEC. SI-CLP co-localizes with stabilin-1 in trans-Golgi network in alternatively activated macrophages. Mechanism of intracellular trafficking of SI-CLP was investigated in primary human alternatively activated macrophages and in H1299 stably transfected with Flag-tagged SI-CLP. In human stabilin-1 positive macrophages major site of SI-CLP localisation was found to be CD63+ lysosomes. Treatment of these macrophages with stabilin-1 siRNA resulted in significantly decreased sorting of SI-CLP in lysosomes. FLAG-SI-CLP in H1299 cells is miss-sorted in globular nuclear structures. Over expression of recombinant stabilin-1 in H1299 stably transfected with Flag-tagged SI-CLP revealed that satbilin-1 mediates re-localisation of SI-CLP from nuclear compartment to the cytoplasmic compartment. Deletion of GGA-binding motif in stabilin-1 resulted in reduced sorting activity of stabilin-1. Thus, stabilin-1 is responsible for intracellular sorting of SI-CLP into lysosomal secretory pathway. Next, SI-CLP was found to be secreted by IL4-stimulated human macrophages in culture conditions, while presence of dexamethasone resulted in accumulation of SI-CLP inside of macrophages.

To investigate biological function of SI-CLP, recombinant SI-CLP-His was expressed and purified from baculovirus-insect cell expression system using Ni-NTA affinity chromatography. Sugar binding studies with cell lysates of alternatively activated macrophages as well as in vitro translated ^{35}S-labelled-SI-CLP and recombinant SI-CLP revealed that it specifically binds to heparin type-I, heparin type-II and chitin.

Expression of SI-CLP was detected in bronchoalveolar lavage samples obtained from patients with chronic inflammatory disorders of the respiratory tract and in peripheral-blood leukocytes from these patients as well as healthy donors. The ability of 1C11 mAb to detect SI-CLP in these conditions will facilitate the investigation of the role of SI-CLP in human diseases.