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Mechanism of stabilin-1 mediated intracellular trafficking

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Stabilin-1 is a type I transmembrane protein selectively expressed on subpopulations of macrophages and non-continuous endothelial cells both in healthy tissues and in pathology including tumor progression and inflammation. As a unique scavenger receptor, stabilin-1 is involved in two intracellular trafficking pathways, receptor-mediated endocytosis and shuttling between the endosomal compartment and trans-Golgi network (TGN). Stabilin-1 mediates endocytosis of acLDL, the matricelluar protein SPARC, and hormone placental lactogen (PL). At the same time, stabilin-1 mediates transport of endogenous chitinase-like protein SI-CLP from the TGN to the endosomal/lysosomal system. Stabilin-1 interacts with the monomeric clathrin adaptors, GGAs through two motifs at its cytoplasmic tail, a classical DXXLL-type motif (DSSLL) and an acidic cluster (EDDADDD). In the present work, the role of GGA-binding sites within cytoplamic tail of stabilin-1 in its endocytic and intracellular sorting function was investigated. Expression constructs for full length stabilin-1 with deleted GGA binding sites DSSLL, EDDADDD as well as double deletion for both sites were created. Effect of the deletions on the endcoytic function of stabilion-1 was examined in CHO cells stably transfected with wt stabilin1-as well as stabilin-1 mutants. Using flow cytometry and confocal microscopy it was demonstrated that deletion of GGA binding sites does not affect either stabilin-1 surface expression or endocytosis of stabilin-1 ligands acLDL, SPARC, PL as well as a-stabilin-1 mAb MS1. However, deletion of EDDADDD but not DDSLL resulted in impaired transport of SPARC to the late endosomal compartment. In addition, it was found that SPARC can be targeted not only to the lysosomal degradation, but also to the degradation proteasomes in stabilin-1-positve macrophages. Overexpression of stabilin-1 wt and mutants in H1299 cells stably transfected with SI-CLP revealed that only wt stabilin-1 is able to perform intracellular sorting of SI-CLP, while all stabilin-1 mutants with deleted GGA binding site were deficient in this function. In conclusion, the results of the present study demonstrate for the first time that both GGA interacting sites are required for the cooperative action during intracellular sorting of SI-CLP performed by stabilin-1. Moreover, GGA-binding EDDADDD site is specifically required for the efficient stabilin-1-mediated targeting of SPARC to the late endocytic pathway.