Analysis of ESCRT complex member CHMP4B interactomes in metabolic stress

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Autophagy is a cellular process of lysosome-dependent degradation and recycling of cytoplasmic components. It is regulated by extra- and intracellular signals to maintain homeostasis and can serve as an adaptative strategy in response to cellular stress. At the molecular level, autophagy regulation mainly relies on the mechanistic target of rapamycin complex 1 (mTORC1), which integrates environmental cues and controls the balance between anabolic and catabolic processes. Characterization of autophagy signaling network by quantitative phosphoproteomics and by siRNA screen revealed a complex interplay between mTORC1 and the SCY1-like protein 1 (SCYL1). Further analysis in our laboratory showed that SCYL1 was also a mTORC1 phosphorylation target, and analysis of the SCYL1 interactome by IP-MS showed an increased interaction with the charged multivesicular body protein 4B (CHMP4B) after autophagy induction.

During this work we explored the role of CHMP4B in autophagy and its interaction with SCYL1. For this purpose, a lentiviral transduction system was used to produce cell lines expressing CHMP4B fused to an HA tag in wild-type and *SCYL1* knock-out MCF-7 cells. After autophagy induction, CHMP4B interactomes of SILAC-labeled cells (stable isotope labelling by amino acids in culture) were obtained by immunoprecipitation and analyzed by quantitative mass spectrometry. SCYL1 was not found in CHMP4B interactomes, suggesting an indirect, possibly vesicle-mediated, interaction. However, an impact on CHMP4B-HA interactome caused by *SCYL1* deletion has been observed. This effect is possibly due to a disorganization of the endomembrane system, previously observed in our laboratory.

This work provides novel insights into ESCRT machinery functions and into its interplay with SCYL1, opening new paths to understand vesicular trafficking in cancer cells.

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