Abstract

Regulated intramembrane proteolysis is an interesting process involved in a multitude of cellular pathways. Enzymes which catalyse this are termed intramembrane proteases (IMPRs), cleaving proteins passing through the membrane within their transmembrane domain. Rhomboid proteases are serine IMPRs. They are widely distributed among organisms and evolutionarily conserved, but despite many efforts, their physiological roles are largely unexplored. RHBDL4 is a mammalian rhomboid protease localised to the endoplasmic reticulum. It is involved in the development of colorectal cancer, which makes it an important focus of research, but its physiological function is not well understood. In order to explore it, I established and employed a proximity proteomics approach, termed APEX2. It is based on biotinylation of proteins in the spatial proximity of the target in the physiological environment of intact living cells. Labelled proteins are subsequently purified, identified and quantified by mass spectrometry. Exploring the physiological vicinity of RHBDL4, its interaction partners and substrates can be revealed and the detailed subcellular compartment, where RHBDL4 resides, can thus be inferred. During three independent experiments in HCT116 cell line, three proteins emerged repeatedly in the RHBDL4 proximity proteome: ANXA7, ITGB1 and PLIN3, indicating that RHBDL4 may be involved in intracellular trafficking and lipid metabolism.

Key words: intramembrane proteolysis, rhomboid protease, RHBDL4, endoplasmic reticulum, proximity proteomics, APEX2