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Functional analysis of *N*-glycans in the quality control of proteins.

In the present study, we analyzed the roles of *N*-glycans in the quality control of glycoproteins in mammals. Newly synthesized proteins acquire their native conformations, and are transported to the organelles where they function. In the endoplasmic reticulum (ER), a large number of secretory and membrane proteins are synthesized. Chaperone proteins and lectins in the ER assist the folding of proteins, a mechanism called as the quality control of proteins. *N*-glycans attached to the glycoproteins are processed and recognized by specific lectins, acting as the tag for protein folding and ERAD (ER-associated protein degradation). Recent studies have revealed that MRH (mannose 6-phosphate receptor homology) domain containing lectins in the ER control the ERAD by binding to specific *N*-glycans. Mammals have two homologues of OS-9 and XTP3-B/Erlectin that belong to this family of lectins. However, the functional difference between OS-9 and XTP3-B remains elusive.

We have purified XTP3-B MRH domains as a fusion protein with bacterial trigger factor. *N*-glycan structures that recombinant XTP3-B MRH domains recognize were examined by frontal affinity chromatography analysis (collaboration with Dr. K. Kato and Dr. Y. Kamiya, Okazaki Institute for Integrative Biosciences). We also evaluated the association of XTP3-B with misfolded glycoproteins in the cells. Effect of XTP3-B on the degradation of a model ERAD substrate α1-antytrypsin null (Hong Kong) (NHK) was investigated by the pulse-chase experiments in RNAi-treated cells. In the ER membrane, there is a large ERAD complex that contains HRD1-SEL1L ubiquitin-ligase complex. Association of endogenous XTP3-B with the ERAD complex was analyzed using sucrose density gradient centrifugation in combination with siRNA (small interfering RNA)-mediated knockdown of endogenous proteins.

We found that C-terminal MRH domain of XTP3-B had lectin activity and recognized M9 ($Man_9GlcNAc_2$) glycans by frontal affinity chromatography. In cells treated with an α1,2-mannosidase inhibitor kifunensine, XTP3-B preferentially bound to NHK bearing M9 glycans. The association was diminished by introducing a mutation into one of the amino acid residues conserved in the MRH domains. NHK degradation is inhibited in cells treated with kifunensine. However, NHK became degraded when endogenous XTP3-B was knocked-down by siRNA. We also elucidated that XTP3-B is unstable, and that all the endogenous XTP3-B associate with the ERAD complex in the ER membrane through direct interaction with SEL1L. Collectively, we propose that XTP3-B is a stable component of the ERAD complex in the mammalian ER membrane, and inhibits the premature degradation of glycoproteins bearing M9 glycans, that are still on the way of folding. Excess ERAD is toxic to cells, and thus, XTP3-B negatively regulates the glycoprotein ERAD.