“The specificity of substrate recognition and transport in the endoplasmic reticulum-associated degradation system (ERAD)”

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The endoplasmic reticulum (ER) environment is optimal for the biosynthesis of, among others, secretory and lysosomal proteins. Proteins with the correct conformation are transported to their target sites, while misfolded polypeptides are specifically recognized in the ER and transported to the cytosol for proteolytic degradation. This process is called ER-associated degradation (ERAD) and is part of the protein folding quality control system that ensures the proper functioning of eukaryotic cells. The process of ERAD substrate recognition involves EDEM family lectins (EDEM1, EDEM2 and EDEM3), as well as Derlin family proteins (Derlin-1, Derlin-2 and Derlin-3), which are involved in the recognition and translocation of protein substrates across the ER membrane. The recognition of protein substrates by ERAD regulators likely depends on factors such as substrate structure and hydrophobicity.

The aim of the present study was therefore to investigate how the structure and hydrophobicity of selected protein substrates affect their recognition and transport from the ER into the cytosol, with a particular focus on the role of EDEM3 and Derlin family proteins in this process. Various protein substrates were used in this study: plant-derived ricin toxin, β-secretase (BACE457) and amyloid precursor protein (APP). The study was conducted on human embryonic kidney cells (HEK293). Ricin is an unusual substrate of the ERAD process. Instead of proteolytic degradation in the cytosol, its enzymatically active subunit A (RTA) affects the inhibition of protein synthesis. The ricin variants used in the study: RTA P250A, RTA DHF and RTA IHF are characterized by altered secondary structure and decreased and increased hydrophobicity, respectively. Model proteins with abnormal conformations are a key tool in studying the mechanisms of substrate targeting in the ER-associated degradation pathway. Examples of such proteins include human β-secretase variants BACE457, $BACE457_{DHF}$ (a variant with reduced hydrophobicity) and BACE457Δ, which lacks a hydrophobic transmembrane domain, making it an ER luminal protein. The APP protein, whose proteolytic truncation can generate the formation of toxic forms of β-amyloid, contributing to the development of Alzheimer's disease, is also an important research model. In this work, two APP isoforms of different lengths, APP_{751} and APP_{695}, were used.

The study showed that overproduction of EDEM3 protein doubly elevates both ricin P250A cytotoxicity and RTA_{P250A} subunit transport from the ER to the cytosol, while having no effect on the reduction of P250A holotoxin in the ER. The role of EDEM3 in RTA_{P250A} transport was also confirmed under conditions of gene knockdown for this protein. Knockdown of EDEM3 resulted in reduced transport efficiency of this subunit from the ER to the cytosol. In experiments using different variants of the toxin: RTA_{P250A}, RTA_{DHF} and RTA_{IHF}, it was shown that EDEM3 interacts most strongly with hydrophobically altered RTA subunits. Altering the secondary structure of the A subunit of ricin also increases its interactions with EDEM3, compared to the interactions of this lectin with wild-type RTA. In addition, experiments using different forms of β-secretase showed that EDEM3 effectively facilitates the degradation of the BACE457Δ form, lacking the hydrophobic domain, and $BACE457_{DHF}$. It can be concluded that in the binding of ERAD substrates by EDEM3, altered structure and hydrophobicity play important roles, directly affecting the transport of these substrates from the ER to the cytosol. Overproduction of Derlin-1 protein, like increased EDEM3, affects sensitization of HEK293 cells to ricin P250A and increases the transport of RTAP250A into the cytosol. Interestingly, overproduction of Derlin-2 protein has no effect on these processes. This suggests a distinct mechanism of action for Derlin-1 and Derlin-2 proteins in the process of RTA transport with altered secondary structure. The study also shows that overexpression of Derlin family protein genes differentially affects the amount of overproduced forms of APP_{695} and APP_{751}, with increased Derlin-3 protein contributing to a significant reduction in the amount of immature forms of both APP_{695} and APP_{751}. In addition, studies on the effect of EDEM family proteins on the amount of overproduced APP_{751} isoform, especially experiments using the proteasome inhibitor epoxomycin, have shown that EDEM1 is a very important regulator of intracellular APP751 protein metabolism.

In conclusion, the conducted studies demonstrated that both the structure and degree of hydrophobicity of proteins play a key role in their recognition and targeting to the ERAD degradation pathway. These findings may have significant implications for the development of therapeutic strategies, particularly in the context of neurodegenerative diseases such as Alzheimer's disease, as well as in toxin research, potentially contributing to the development of new medical applications.