E3 Ubiquitin Ligase HRD1 regulates the Serotonin Transporter Function

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The serotonin transporter (SERT) is responsible for the termination of serotonergic neural transmission via reuptake of serotonin (5-HT) at nerve terminals. We investigated the involvement of the ubiquitin E3 ligase HRD1 (HMG-CoA reductase degradation protein), which participates in endoplasmic reticulum (ER)-associated degradation (ERAD), in the functional regulation of SERT in order to elucidate the regulation of SERT via its membrane trafficking. We used HEK293 cells and COS7 cells transiently expressing wild-type SERT or a SERT C-terminal deletion mutant (SERT Δ CT), a SERT protein predicted to be misfolded. Studies using HRD1-overexpressing or HRD1-knockdown cells demonstrated that HRD1 is involved in SERT proteolysis. Overexpression of HRD1 promoted SERT ubiquitination, the effect of which was augmented by treatment with the proteasome inhibitor MG132. Immunoprecipitation studies revealed that HRD1 interacts with SERT in the presence of MG132. In addition, HRD1 was intracellularly colocalized with SERT, especially with aggregates of SERT Δ CT in the ER. HRD1 also affected SERT uptake activity in accordance with the expression levels of the SERT protein. These results suggest that HRD1 contributes to the membrane trafficking and functional regulation of SERT through its involvement in ERAD-mediated SERT degradation.