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Entitled

DELINEATING THE CELLULAR MECHANISMS OF ENDOPLASMIC RETICULUM-RETAINED ENDOGLIN MUTANTS CAUSING HEREDITARY HEMORRHAGIC TELANGIECTASIA TYPE 1

by

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Abstract

Endoglin, also known as cluster of differentiation 105 (CD105), is an auxiliary receptor in the TGFβ signaling pathway. It is predominantly expressed in endothelial cells as a component of the heterotetrameric receptor dimers comprising type I, type II receptors and the binding ligands. Mutations in the gene encoding Endoglin (*ENG*) have been associated with hereditary hemorrhagic telangiectasia type 1 (HHT1), a rare autosomal dominant inherited disorder affecting about 1 in 5000-8000 individuals, which is generally characterized by vascular malformations. Secretory and many endomembrane proteins synthesized in the Endoplasmic reticulum (ER) are subjected to a highly stringent protein folding and assembly quality control mechanisms to ensure that only properly folded and assembled proteins are transported forward through the secretory pathway to their final destinations. We have previously demonstrated that some Endoglin variants causing HHT1 are retained in the ER and failed to traffic to their normal localization at the plasma membrane, which suggested the possible involvement of the ER associated protein degradation (ERAD) in their molecular pathology. In this study, we have investigated the detailed degradation routes of Endoglin wild type and two ER-retained mutant variants, P165L and V105D. Stably transfected HEK293 cells expressing wild type (WT) and the two mutants were treated with proteasomal and lysosomal inhibitors to elucidate their cellular degradation pathways and the molecular mechanisms underlying the loss of function phenotype associated with the disease-causing variants. Our results show that WT Endoglin has a relatively short half-life of less than 2 hours and degrades through both the lysosomal and proteasomal pathways, whereas the two disease-causing mutant variants (P165L and V105D) are relatively stable with half-life of more than 16 hours and predominantly degrade through the proteasomal ubiquitin pathway. Furthermore, we have demonstrated that Endoglin variants P165L and V105D are significantly accumulated in the CRISPR-Cas9-generated HEK293 cells deficient in HRD1 E3 ubiquitin ligase; a major ERAD component. Therefore, our results conclusively confirm the involvement of ERAD in the cellular mechanisms of some HHT1 disease-causing missense variants. These findings might pave the way for more in-depth research studies that could open new windows for future therapeutic interventions for patients with HHT1.

We have also investigated if ER-retained HHT1-causing Endoglin variants L32R, C53R, V105D, I271N, P165L and C363Y would exert dominant negative effects by hijacking the wild type allele in the ER. Our results show that these ER- retained Endoglin mutant variants are able to form heterodimers with the wild type protein. Crucially, they exhibit considerable dominant negative effects manifested in significant entrapment of wild type Endoglin within the ER leading to reduced maturation. This suggest that in addition to haploinsufficiency due to mutations in one allele, heterozygous carriers of these ER-retained variants are likely to lose part of the function of the WT allele. These findings may help explain some of the phenotypic heterogeneity amongst HTT1 patients.

Keywords: Endoglin, TGFβ signaling pathway, Hereditary hemorrhagic telangiectasia type 1, ERAD, Endoplasmic reticulum, ER-retained mutant variants.