ERAD contributes to the degradation of Endoglin co-receptor and the development of Hereditary Hemorrhagic Telangiectasia

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Introduduction

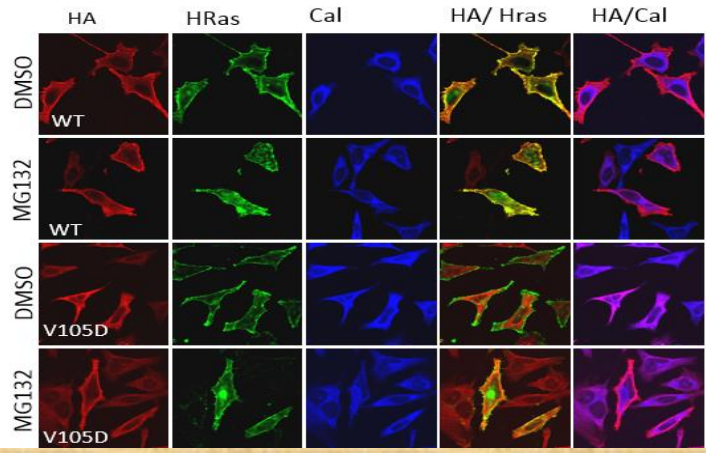
Hereditary hemorrhagic telangiectasia type 1 (HHT1), also known as Rendu-Osler-Weber syndrome, is an autosomaly dominant inherited disease that is generally characterized by vascular malformation and fragility. HHT1 has been associated with mutations in the TGFβ co-receptor Endoglin, encoded by *ENG* gene.

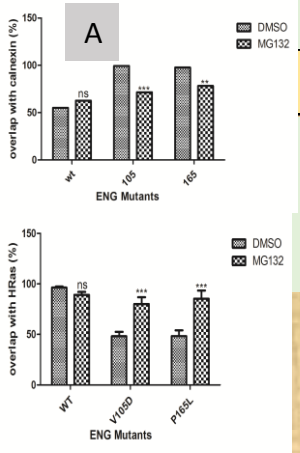
Aims & objectives: Investigate the degradation pathway of wild type (WT) and mutant Endoglin retained in the ER in order to elucidate the exact molecular mechanisms underlying the loss of function phenotype associated with this disease.

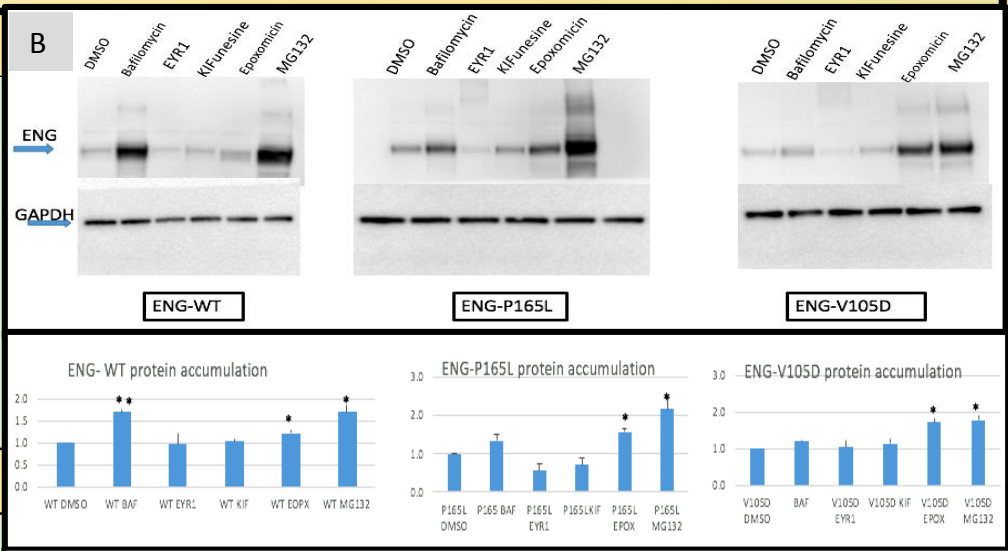
Methodology

Stable HEK293 cell lines harboring the HA-tagged ENG-WT and mutant variants p.P165L and p.V105D were generated. Cycloheximide chase assay was used to determine the cellular half-life of ENG-WT and mutant variants. To delineate the degradation pathways, HEK293 cells were incubated with ERAD, proteasomal, or lysosomal inhibitors (Eeyarestatin I, and Kifunesine /Bafilomycin, chloroquine/ MG132, Epoxomycin, respectively). Western blot was performed to measure protein accumulation level. HEK293$^{(HRD1\text{-}KO)}$ cell lines were generated using (Origene) HRD1 KN.2 kit for CRISPR Cas9 gene Knock out (KO).

Results







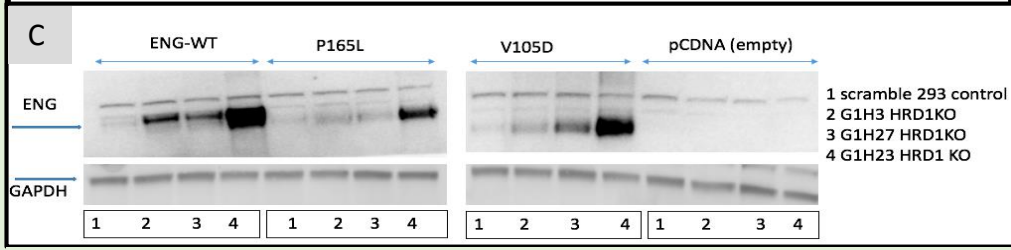


Figure (A) shows enhanced cell surface expression of mutants ENG after proteasomal inhibition. Figure (B) shows Endoglin-WT accumulation after treatment with Bafilomycin & MG132, while Endoglin mutant variants significantly accumulate after treatment with MG132 and epoxomycin. Figure (C)shows Endoglin acumulation in generated HEK293$^{(HRD1\text{-}KO)}$ cell lines.

Conclusions

We demonstrate for the first time the implication of ERAD in the pathogenesis of HHT1 disease through the elucidation of the degradation pathways of both wild type (WT) and disease-causing mutant variants of Endoglin.

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