

REHAM MAHMOUD MOH'D MILHEM

Department of Pathology
College of Medicine and Health Sciences



Dissertation

Title *ELUCIDATION OF THE CELLULAR AND MOLECULAR MECHANISMS OF MISSENSE MUTATIONS ASSOCIATED WITH FAMILIAL EXUDATIVE VITREORETINOPATHY AND CONGENITAL MYASTHENIC SYNDROME*

Faculty Advisor Prof. Bassam Ali

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Abstract

The endoplasmic reticulum (ER), within eukaryotic cells, is a hub for protein folding and assembly. Mis-folded proteins and unassembled subunits of protein complexes are retained in the ER and degraded by a process termed endoplasmic reticulum associated degradation (ERAD). Frizzled class receptor 4 (FZD4) and muscle, skeletal, receptor tyrosine kinase (MuSK) are Wnt receptors. These proteins contain the cysteine-rich domain (Fz-CRD) required for dimerization in the ER. Mutations in FZD4 and MuSK genes are known to cause familial exudative vitreoretinopathy (FEVR, an autosomal dominant disease) and congenital myasthenic syndrome (CMS, an autosomal recessive disease), respectively. It was hypothesized that missense mutations within Fz-CRD lead to misfolding of FZD4 and MuSK proteins and consequent ER-retention. Investigating the molecular mechanism of these mutations is important since misfolded protein and ER-targeted therapies are in development. Wild-type and mutants of FZD4 and MuSK were expressed at 37 °C in HeLa, COS-7, and HEK293 cells and their subcellular localizations were investigated by confocal microscopy imaging and glycosidase treatments. Abnormal trafficking was demonstrated in 10 of 21 studied mutants; nine mutants were within Fz-CRD and one was distant from Fz-CRD. These ER-retained mutants were improperly N-glycosylated confirming ER-localization. They were tagged with polyubiquitin chains confirming targeting for proteasomal degradation. The half-lives of wild-type MuSK and P344R-MuSK were 90 and 37 min, respectively; the latter half-life improved on incubation with the proteasomal inhibitor MG132. The P344R-MuSK kinase mutant showed around 50% of its in vivo autophosphorylation activity. Trafficking defects in three of the 10 mutants (M105T-FZD4, C204Y-FZD4, and P344R-MuSK) were rescued by expression at 27 °C and by chemical chaperones (2.5-7.5% glycerol, 0.1-1% dithiothreitol sulfoxide, 10 µM thapsigargin, or 1 µM curcumin). Trafficking of wild-type FZD4 was not affected by co-expression with any of the nine ER-retained mutants, suggesting haploinsufficiency as the mechanism of disease. Thus, all nine studied Fz-CRD mutants of FEVR and CMS resulted in misfolded proteins. In contrast, only one of the 12 mutants outside Fz-CRD resulted in ER-retention. These findings demonstrate a common mechanism for diseases associated with Fz-CRD missense mutations. Disorders of Fz-CRD could be amenable to novel therapies that alleviate protein misfolding.

Research Relevance and Potential Impact

Reham Milhem's PhD research resulted in the elucidation of the cellular mechanisms underlying many mutations causing two genetic disorders, namely congenital myasthenic syndrome and familial exudative vitreoretinopathy. These findings are original and are likely to have impact on our understanding of the pathogenesis of these incurable disorders and possibly help in developing novel therapies for them. The results have been published in journals that are in the top 5-10% of their respective fields.