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**“D1080N-IRBP Misfolding and ER Stress in Retinitis Pigmentosa
Modulation and Rescue of Protein Secretion”**

Background: Retinitis pigmentosa (RP) is an inherited retinal disorder characterized by progressive photoreceptor degeneration. Interphotoreceptor retinoid-binding protein (IRBP) is secreted by photoreceptors into the interphotoreceptor matrix, where it supports retinoid transport and photoreceptor survival. A homozygous D1080N mutation in IRBP was identified in patients with autosomal recessive RP, yet the mechanism linking this mutation to photoreceptor degeneration remains incompletely understood. Previous studies demonstrated that D1080N-IRBP is poorly secreted and induces endoplasmic reticulum (ER) stress, raising the question of whether the secretory defect is absolute or can be modified by improving protein-folding conditions. We therefore examined how D1080N affects IRBP trafficking and ER stress and whether mutant IRBP secretion can be partially restored.

Methods: HEK293T cells expressing wild-type (WT) or D1080N-IRBP were cultured under varying fetal bovine serum (FBS) concentrations and reduced temperatures (26°C and 30°C). Additional cultures were treated with the chemical chaperone 4-phenylbutyrate (PBA), an FDA approved medication. Secreted IRBP and unfolded protein response (UPR) markers (ATF4 and cleaved ATF6) were measured by immunoblotting. Subcellular localization, including colocalization with the ER marker KDEL, was assessed by immunocytochemistry.

Results: Unlike WT-IRBP, D1080N-IRBP accumulated in the ER and showed increased colocalization with KDEL. Cells expressing the mutant protein had elevated ATF4 and cleaved ATF6, indicating UPR activation, and showed impaired secretion. Incubation at reduced temperature increased extracellular D1080N-IRBP, demonstrating partial restoration of secretion, while changes in FBS concentration modulated extracellular mutant IRBP levels. PBA improved the intracellular distribution of D1080N-IRBP and partially restored its secretion.

Conclusion: D1080N-IRBP misfolds, accumulates within the ER, activates the UPR, and exhibits impaired secretion, providing a potential mechanism linking the D1080N mutation to photoreceptor degeneration in RP. Partial restoration of mutant IRBP secretion by reduced temperature and PBA, together with modulation by extracellular conditions, demonstrates that the secretory defect is modifiable rather than absolute. These findings identify protein folding and intracellular trafficking as potential therapeutic targets in D1080N-IRBP-associated RP.

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