Effects of S1P Mutation on ER Stress and Cholesterol Synthesis Markers in Human Epithelial Cells

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INTRODUCTION

Site-1 Protease (S1P) is a Golgi-resident enzyme required for activation and subsequent nuclear localization of several major transcription factors. A 24-year-old female patient with a de novo single point mutation in S1P presented with a complex phenotype of gut hypomotility, abnormal optic nerves, and polycystic ovarian syndrome. Furthermore, this patient suffers from phenotypes related to skeletal muscle dysfunction, resulting in decreased ability to perform physical exercise due to unusual post-exercise pain, fatigue, nausea, and vomiting. This phenomenon has been described in the literature to manifest from myoedema and the breakdown of muscle that releases intracellular proteins into the blood, or rhabdomyolysis.

Exomic sequencing revealed a heterozygous amino acid substitution (P1003S) in the transmembrane domain of S1P. Previous research has shown that S1P plays an integral role in cleaving the transcription factors ATF6 and SREBP2. ATF6 is a key protein leading to the expression of ER stress genes while SREBP2 is the major protein leading to the activation of cholesterol synthesis genes.

RESULTS

Figure 2. MBTPS1mut is a gain of function mutation for ER Stress ATF6 tested with 1mg/mL tunicamycin over a 12 hour time course [A] GRP78 [B] CHOP [C] XBP1

The goal of this study was to characterize the mutant S1P protein by assessing protein activity and localization. Over-expression of mutant S1P in a lipid and cholesterol auxotrophic S1P-null cell line rescued the dependence on exogenous lipids and sterols similar to null cells expressing wild-type S1P. Furthermore, induction of ER stress with tunicamycin showed a heightened expression of ATF6 target genes in mutant S1P patient fibroblasts relative to control patient cells. A similar elevated response in SREBP2 target genes was also observed when the SREBP2 pathway was stimulated in the mutant fibroblasts. In addition, EndoH and PNGase sensitivity assays showed that localization of mutant S1P to the Golgi was not impaired. In the crystal violet assay, mutant S1P growth was not hindered by the lack of cholesterol in the media and grew more than the WT. This initial characterization demonstrated that the de novo mutation produces a gain-of-function phenotype and that the mutation does not disrupt proper localization of the protein. This is the first known case of S1P mutation in humans and it is unknown how many harbor similar mutations of the S1P protein, critical for sterol homeostasis.

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