To study the effect of mutation on the γ subunit of the COP1 protein complex

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ABSTRACT

Endoplasmic Reticulum bears ER resident chaperones, like Protein Disulphide Isomerase (PDI), Binding Immunoglobulin Protein (BiP), calnexin, calreticulin that assist in protein folding and prevent nascent protein aggregation in the ER. Protein trafficking from the ER to golgi involves transfer of ER resident chaperones to the golgi, these chaperones bear a C-terminal KDEL sequence which is identified by the KDEL receptor (KDELR) present in the golgi apparatus. A di-lysine or di-arginine motif, close to the KDELR recruits the COP1 vesicle protein, which mediates the retrograde transport of the ER resident chaperones back to the ER. The COP1 protein is composed of a coatomer, a heptameric complex containing α, β, β′, γ, δ, ε, and ζ. A mutation in the γ subunit of the COP1 protein complex inhibits the binding of the KDELR by the coatomer, this ceases the retrograde trafficking of ER chaperones between the ER and golgi by the COP1 protein complex which leads to ER stress (1).

ER stress due to a missense Copg1^{K652E} mutation can be studied by Sangner sequencing in the affected patients. To minimize target effects, CRISPR design tool was used to synthesize sgRNA. To quantify the retrograde transport, a golgi marker GM130 was used for colocalization of the COP1 cargo protein followed by confocal microscopy. Binding of the coatomer to different proteins can be studied by GST assays followed by analysis by SDS-PAGE followed by immunoblotting and Coomasie staining. Time-Correlated Single-Photon Counting Fluorescence lifetime image microscopy can be used to study the interaction between mutant COP1 and KDELR. ELISA was used to study BiP secretion from B cells and cytotoxic assays for T cells and NK cells were also performed.

Mutant B and T cells exhibit ER stress due to absence of chaperones which leads to decreased IgG secretion due to IgG misfolding in the ER. Increase in the serum TNF-α, IFN-γ, and IL-6 leads to conditions like cachexia where the patient exhibits body weight loss and loss of muscle protein, T-cell lymphophenia and plasmacytosis. Increase in ER stress leads to the activation of NF-Kb and Ap-1 pathways which leads to secretion of high amount of inflammatory

cytokines, this can further increase ER stress which can cause apoptosis of T cells and B cells. Increased apoptosis is observed in the T cells due to the high level of expression of CHOP which a pro-apoptotic transcription factor. Thus, mutation in the γ subunit of the COP1 protein complex is the first known primary immunodeficiency disease. It reduces patients immunity towards infections and can ultimately result into death. This condition, caused by the mutation can be reversed by treating the patient with Tauroursodeoxycholic Acid (TUDCA). Reversal of the conditions upon treating with TUDCA also proves that the immunodeficiency is caused due to ER stress (2).

Keywords : COP1, Chaperones, Immunodeficiency, Mutant, Stress

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