Role of HIV-1 Nef Lysine Residues 4 and 7 in the Interaction with Calnexin and Inhibition of ABCA1 Activity

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ABSTRACT
Patients infected with HIV are at increased risk of developing atherosclerosis, a result due partly to the functional impairment of cellular cholesterol transporter, ATP-binding cassette transporter A1 (ABCA1). This transporter mediates efflux of cholesterol from cells to high density lipoprotein, thus allowing cells to maintain normal levels of cholesterol. When ABCA1 function is inhibited, cells, and in particular macrophages, accumulate cholesterol, resulting in formation of atherosclerotic plaques. The HIV-1 protein, Nef downregulates ABCA1 and inhibits the activity of this protein, thereby reducing cholesterol efflux and promoting atherosclerosis, but the molecular mechanism by which Nef inhibits ABCA1 is unknown.

Previous studies demonstrated that Nef blocks interaction between ABCA1 and endoplasmic reticulum chaperone, calnexin. The calnexin-ABCA1 interaction is required for the functionality of ABCA1, as it was shown that cholesterol efflux was inhibited when calnexin expression was knocked down. It was shown that Nef binds to calnexin, and this binding is required for inhibition of ABCA1 activity.

Bioinformatic analysis of Nef-calnexin interaction identified lysine residues at positions 4 and 7 of the Nef amino acid sequence as critical for interaction with calnexin. My project is to perform site-directed mutagenesis of Nef to introduce single and double mutations replacing lysines with alanines at positions 4 or 7. Experiments are ongoing to express these Nef mutants in 293T cells and analyze the contribution of each amino acid to Nef interaction with calnexin and down-regulation of ABCA1. These studies will contribute to the development of drugs targeting cholesterol deregulation caused by HIV-1 Nef.

Key Words: Nef, ABCA1, Calnexin, Cholesterol
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