COMPOUNDS INHIBITING NEF-CALNEXIN INTERACTION

CROSS-REFERENCE OF RELATED APPLICATION

[0001] This application is a U.S. National Stage Application under 35 U.S.C. § 371 of PCT/US2017/013236, filed on Jan. 12, 2017, the entire content of which is hereby incorporated by reference, and claims priority to U.S. Provisional Application No. 62/277,720 filed Jan. 12, 2016; the entire contents of all of which are hereby incorporated by reference.

FEDERAL FUNDING BY U.S. GOVERNMENT

[0002] This invention was made with Government support under Grant Nos. R21 AI114471, R01 HL101274 and R21 AI108533 each awarded by The National Institutes of Health (NIH). The U.S. Government has certain rights in the invention.

BACKGROUND

1. Technical Field

[0003] The field of the currently claimed embodiments of this invention relates to compounds and methods for restoring or preserving cholesterol efflux in a cell infected with Human Immunodeficiency Virus (HIV) by preventing or decreasing an interaction between Negative Regulatory Factor (Nef) protein and Calnexin protein, and methods for screening for such compounds.

2. Discussion of Related Art

[0004] Highly active anti-retroviral therapy (HAART) has transformed treatment of the HIV disease changing prognosis from acutely lethal to chronic illness, and lifespan of HIV-infected subjects approximates that of uninfected individuals. However, HAART does not cure HIV, and chronic HIV infection is associated with a number of co-morbidities, such as premature atherosclerosis and cardio-vascular disease (37). An essential component in pathogenesis of cardiovascular disease in HIV-infected subjects is HIV-associated dyslipidemia, which is caused both by drugs used to treat HIV infection and by the effects of HIV itself on cholesterol metabolism (38).

[0005] HIV-1 infection, via activity of viral protein Nef, impairs cholesterol efflux mediated by the cholesterol transporter ATP-Binding Cassette A1 (ABCA1) (1). ABCA1 is the main cellular cholesterol transporter regulating delivery of cellular cholesterol to extracellular acceptor, apolipoprotein A-I. Studies in animal models demonstrated that this activity of Nef may be responsible for hypoalphalipoproteinemia and high risk of atherosclerosis observed in HIV-infected subjects (2-4). Recent studies identified calnexin, an integral endoplasmic reticulum (ER) membrane lectin-like chaperone, as a key player in the mechanism of Nef-mediated inhibition of ABCA1 and cholesterol efflux (5). Calnexin (CNX) and its homologue calreticulin (CRT) regulate folding and maturation of newly synthesized glycoproteins by engaging them in a CNX/CRT cycle (6).

[0006] ABCA1 is a highly glycosylated protein (7). Although no evidence for the role of CNX in ABCA1 biogenesis is available, two well-studied ABC transporters, ABCC7 (also known as cystic fibrosis transmembrane conductance regulator, CFTR) and ABCB1 (also known as multidrug resistance protein 1 or P-glycoprotein 1), interact with CNX, and folding mutants of these transporters are retained within the ER by CNX and eventually degraded (8, 9). Importantly, ABCC7 and ABCB1 mutants that escape CNX binding do not achieve mature glycosylation and these mutations result in reduced transporter function (8, 9). A recently published study demonstrated that ABCA1 interacts with CNX, and reduction of CNX expression by RNAi resulted in a significant decrease in functional activity of ABCA1, evidenced by reduced cholesterol efflux to ABCA1-specific acceptor apoA-I (5). It was also shown that Nef impairs interaction between ABCA1 and CNX, and this effect of Nef is essential for inactivation and downregulation of ABCA1 (5). Importantly, inhibition of ABCA1-calnexin interaction by Nef is specific, as interaction between ABCA1 and two other proteins, dystrophin and serine palmitoyltransferase, shown previously to bind ABCA1 (10), was not affected. Also not affected was the interaction between calnexin and HIV-1 envelope glycoprotein, gp160; in fact this interaction was even enhanced by Nef (5). These findings suggested that Nef modulates activity of calnexin, but the mechanism of this effect and molecular details of Nef/calnexin interaction remained unknown. Moreover, it was unclear whether the interaction between Nef and calnexin is direct, making screen for inhibitory compounds difficult.

[0007] Calnexin is a 592-amino acid Type I transmembrane protein composed of three parts: a lumenal fragment consisting of a globular β-sandwich domain responsible for the interaction with carbohydrates and a proline-rich tandem sequence repeat domain (the P domain) involved in protein-protein interactions, a transmembrane domain, and a cytoplasmic domain of 90 residues (11, 12). The cytoplasmic tail of calnexin can undergo phosphorylation and palmitoylation which regulate calnexin association with a number of proteins and protein complexes that influence functional activity of this chaperone (13-18). For example, palmitoylation of the C-tail of calnexin mediates its association with the ribosome-translocon complex, which is essential for the ability of calnexin to capture its client proteins as they emerge from the translocon (18). Ribosome association of calnexin is also regulated by phosphorylation on Ser534 and Ser544 by casein kinase 2 and on Ser563 by protein kinase C/proline-directed kinase (11). In addition, phosphorylation at Ser563 has been shown to play essential role in quality control function of calnexin (15). Therefore, the C-tail of calnexin may play a functional role regulating activity of the chaperone both directly, by affecting ER lumenal events involving calnexin, and indirectly, via modification of calnexin localization in the ER.

SUMMARY

[0008] An embodiment of the invention relates to a method for restoring or preserving cholesterol efflux in a cell infected with Human Immunodeficiency Virus (HIV) comprising delivering to the cell an effective amount of a composition or formulation comprising a small molecule. The small molecule prevents or decreases an interaction between a Negative Regulatory Factor (Nef) protein and a Calnexin protein.

[0009] Another embodiment of the invention relates to a method for treating or preventing atherosclerosis in a subject infected with HIV comprising administering to said subject