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Studies on HIV-1 Virion Infectivity Factor

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Abstract

Virion Infectivity Factor (Vif) protein of human immunodeficiency virus type 1 (HIV-1) is essential for the productive viral infection of primary human CD4 T lymphocytes and macrophages. Recently, it has been reported that Vif overcomes the HIV-inhibitory effects of the cellular factor CEM15/APOBEC3G, which has cytidine deaminase activity. Using the yeast two-hybrid system, our laboratory previously reported the identification of a Vif-interacting ring finger protein called Triad 3 (renamed Triad 3au in this study), from a human leukocyte cDNA library. The full-length cellular protein homologue of Triad 3 has been recently identified as the zinc finger protein inhibiting NF*k*B (ZIN). In this study, biological characteristics of Triad 3au/ZIN were investigated. Sequence analysis indicated that Triad 3au protein contains all 4 major ring-like motifs of ZIN. RNA was extracted from A3.01 cells and RT PCR was then performed using the ZIN gene specific primers. A single product of 1,464 bps was obtained and subsequently confirmed as the full-length coding region of ZIN by sequence analysis. GST fusion protein pull-down experiments confirmed that overexpressed ZIN binds to purified Vif *in vitro* suggesting a direct interaction between ZIN and Vif. Next, in studies of co-transfected human 293T cells, Triad 3au/ZIN and Vif were shown to interact using co-immunoprecipitation and confocal microscopy demonstrated co-localisation of Triad 3au and Vif in cytoplasm and membrane while co-localisation of ZIN and Vif in nuclei.

To test the biological relevance of the Vif-ZIN interaction, infectious HIV-1 NL4.3 virus stocks were produced in 293T cells expressing Flag tagged ZIN or Flag tag

alone from transfected plasmids. The virus stocks produced in the presence of exogenously expressed ZIN were less infectious in both single-cycle infectivity assay and end-point titration compared with virus produced in the absence of exogenous ZIN. Real Time PCR analyses showed that cells infected with HIV NL4.3 virus stocks produced in the presence of exogenously expressed ZIN, showed reduced levels of early viral DNA synthesis. This reduction in viral reverse transcription and the reduction in single-cycle viral infectivity were shown to be dependent on the presence of Vif in the virus producer cells. The possible mechanisms by which presence of ZIN in producer cells reduces HIV-1 reverse transcription and replication in target cells are discussed.