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Commentary

Influence of Nuclear Factors in HIV-1 Transcription

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DESCRIPTION

Significantly lowering the mortality and morbidity of infected people, Highly Active Anti-Retroviral Therapy (HAART) has transformed the treatment of HIV-1 infection. Nonetheless, only a small number of underdeveloped nations, where 95% of those with HIV and AIDS reside, have access to antiretroviral. As a result, there is a pressing demand for less expensive medicines. The HIV-1 long terminal repeat contains *cis*-regulatory components that interact with host cellular transcription factors to cause HIV-1 replication. The core enhancer, which has two binding sites for nuclear factor B (NF- B) that are essential in the control of HIV transcription, is one of the functional areas present in the HIV-1 Long Terminal Repeat (LTR) promoter that are necessary for transcriptional activation. Both sites are active during transcription; a point mutation at one of the binding sites eliminates this activation.

The ancient medicinal herb *Withania somnifera*, commonly known as Ashwagandha or Indian Winter Cherry, has been used for millennia in Indian Ayurveda medicine to treat a variety of ailments. *W. somniferous* roots and leaves are frequently recommended for the treatment of tumours, inflammation, arthritis, asthma, and hypertension. Withaferin A (WA), one of the earliest members of the group to be isolated and to exhibit important pharmacological effects, such as anti-tumor, anti-inflammatory, and immunomodulatory action, is among the biggest and structurally most diverse collection of withanolides found in *somnifera*. Several human leukemic cell lines show substantial inhibition of growth in the presence of WA, and human breast cancer cells experience mitotic arrest as a result. Recent research has demonstrated that *W. somnifer*a extract, particularly its primary component. The wild-type (GGGACTTTCC) HIV-1 HXB2 LTR (-453 to+80) controls the *luciferase* reporter gene, which is included on the pLTR-luc plasmid. A simple promoter and five consensus NF-B-binding regions that regulate the *luciferase* gene make up the commercial pNF-B-luc molecular construct. Cell signaling technologies was used to obtain the anti-IB- and anti-actin antibodies. Santa Cruz Biotechnology sold the anti-NF-B-p50 (H119) and anti-NF-B-p65 (C20) antibodies.

While the 1G5 cell line was provided by the Acquired immunodeficiency syndrome (AIDS) reagent program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), the parental lymphoid T cell line was obtained from the American Type Culture Collection (ATCC). An HIV-1 Long Terminal Repeat (LTR)-driven *luciferase* gene was stably transfected into cells to create the clonal cell line. The cells were cultivated in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% heat-inactivated foetal bovine serum and kept at 37°C in a 5% CO_2 humidified environment.

Prior to infection, the amount of infectious proviral content in cells was measured by quantitative Polymerase Chain Reaction (qPCR), and infection experiments were carried out using a quantity equal to 1 virus/cell. In order to allow pre-infection, virus stock was introduced directly to cells in the smallest volume of complete media. This was done for 1 hour at 37°C.

The cells were then kept at 37°C for 48 hours. As previously mentioned, viral reporter expression was assessed. In a nutshell, cells were treated twice with ice-cold Phosphate-Buffered Saline (PBS) before 1/5 of the cells were utilized for the qPCR proviral content quantification test and 4/5 were used for the *luciferase* experiment.

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