Research Paper Medical Science

The HIV Biology of pro-viral HIV Gene Expression for Diagnosis and Treatment

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ABSTRACT

Bringing the global Human immunodeficiency virus (HIV) epidemic under control will require more effective approaches to prevent the spread of the retrovirus, as well as broader use of existing and future antiretroviral drugs. These interventions must be applicable in the developing world, where HIV has the most severe impact. Understanding the dynamic interplay of HIV with its cellular host provides the biological basis for controlling the epidemic. This report reviews current understanding of the HIV expression with particular attention to the interactions between viral proteins and cellular machinery, and highlights promising future points of attack.

KEYWORDS HIV, Tat, Rev, cellular co-factors

Introduction:

Human immunodeficiency virus (HIV) is retro-virus that are the primary etiological agents of acquired immunodeficiency syndrome (AIDS), respectively a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive.1,2 Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.3 Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.4 HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by $CD8^+$ cytotoxic lymphocytes that recognize infected cells.5,6 When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. They contain characteristic regulatory genes in addition to gag, pol, env genes, which are found in all retroviruses. Among them, Rev gene of HIV is thought to regulate viral mRNA transport, processing, or stability at post-transcriptional stages. Recent studies revealed the functional similarity, and predominant nuclear localization of these gene products. We have originally reported that the arginine-rich cluster in Rev and Tat of HIV acts as an autonomous signal capable of directing the hybrid protein to the cell nucleolus.7

Nuclear Targeting Signal of Rev and Tat

Regulator of Expression of Virion (Rev) of HIV regulates splicing of viral RNAs, Rev is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the Rev Response Element (RRE) RNA element. Inspite of these similarities, Rev protein shares little homology in their primary structure. However, Rev possesses a nuclear targeting signal (NTS) i.e., NTS of Rev in the middle-portion of its amino-acid sequence. They consist of 16 or 19 amino acid residues characterized by clustered basic amino acid residues. These NTSs have the ability to accumulate β-galactosidase into nucleoli when fused to its amino terminal region. Deletion experiments of Rev gene revealed that they are essential for the nuclear-localization and the function of Rev protein. Mutant genes derived from Rev of HIV-I were constructed in order to investigate the functional aspect of the NTS in Rev. This non-functional NTS mutant protein inhibited the function of Rev. To give insight into the nature of this inhibitory effect, we examined the sub-cellular localization of wild type Rev using anti Rev antibody. In the presence of the product, the intact Rev was excluded from cell nucleolus in transfected cells. In contrast, Rev whose NTS region was substituted with the NTS of Rev, located predominantly in cell nucleolus and functioned like the wild type protein in the Rev assay system.

It has been proposed that the Trans-Activator of Transcription (Tat) protein accelerates the rate of virus production at one or more levels of control, such as RNA transport, RNA stability, and translation efficiency, as well as having effects on transcription. Tat possesses a stretch of basic amino acid residues which is highly conserved among various HIV-1 isolates, Rev is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. To examine whether the basic region of' Tat is crucial for nuclear and nuclear migration, we constructed recombinant plasmids encoding for hybrid proteins with Tat at the amino terminus and β-galactosidase at the carboxy terminus. We also used a vaccinia virus (VV) expression vector system to archive a high level of transient gene expression in the VV-infected cells. In this VV system, Tat was also localized predominantly in the nucleus.

Replication and transcription

Shortly after the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule.8 The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the antisense cDNA.9 Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called integrase.8 This integrated viral DNA may then lie dormant, in the latent stage of HIV infection.8 To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-kappa B (NF-κB), which is upregulated when T-lymphocytes become activated.10 This means that those cells most likely to be killed by HIV are those currently fighting infection.

During viral replication, the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces. These small pieces are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat and Rev. As the newly produced Rev protein accumulates

in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced. At this stage, the structural proteins gag and env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to gag protein and is packaged into new virus particles.11 HIV-1 and HIV-2 appear to package their RNA differently; HIV-1 will bind to any appropriate RNA, whereas HIV-2 will preferentially bind to the mRNA that was used to create the Gag protein itself. This may mean that HIV-1 is better able to mutate (HIV-1 infection progresses to AIDS faster than HIV-2 infection and is responsible for the majority of global infections).

Diagnosis and treatment.

HIV testing and counselling (HTC) services have helped millions of people learn their HIV status, and for those testing positive, learn about options for long term care and treatment. In 2012, WHO estimated that 118 million people in 124 low- and middle-income countries received HIV testing and counselling in the past 12 months and learned their results.12 HIV self-testing is a process whereby a person who wants to know his or her HIV status collects a specimen, performs a test and interprets the test result in private.13 HIV self-testing does not provide a definitive diagnosis; instead, it is a screening test for the presence of HIV-1/2 antibodies or the HIV-1 p24 antigen. Any positive HIV result must be confirmed by a health worker in accordance with national testing algorithms.14,15 HIV self-testing enables individuals to test themselves for HIV in private. By providing an opportunity for people to test themselves discreetly and conveniently, HIV self-testing may provide people who are not currently reached by existing HIV testing and counselling (HTC) services with information about their HIV status.

Adolescents (10-19 years) continue to be vulnerable to HIV infection.16 All adolescents are vulnerable to HIV due to the physical and emotional transitions, and potentially heightened risk-taking behaviour, inherent to this period of life. This is particularly true for adolescents who live in settings with a generalized HIV epidemic-especially girls in sub-Saharan Africa who often face a higher risk of infection than boys-and/or adolescents who are members of key populations at higher risk for HIV acquisition or transmission through sexual transmission and injecting drug use.17 In 2012, an estimated 2.1 million adolescents were living with HIV. Between 2005 and 2012, HIV-related deaths among adolescents increased by 50%, while the global number of HIV-related deaths fell by 30%. This increase in adolescent HIV-related deaths is due primarily to poor prioritization of adolescents in national HIV plans, inadequate provision of accessible and acceptable HIV testing and counselling (HTC) and treatment services, and lack of support for adolescents to remain in care and adhere to anti-retroviral therapy (ART). The update to the Consolidated anti-retroviral (ARV) guidelines will address these transition and treatment issues for adolescents in greater detail and will be available in 2014. 18

There is currently no vaccine against HIV/AIDS. However, there are a number of very encouraging leads towards the development of a HIV vaccine. Thus, a combination of two vaccines, a modified poxvirus expressing HIV antigen and a so-called protein subunit vaccine, was shown to provide over 30% protection against HIV infection in a human clinical trial, the "RV144 protocol", in Thailand, in 2009.19 The current vaccine development effort is therefore two-pronged: to improve on the results of the RV144 protocol, in trials planned in Thailand and South Africa and to develop a whole range of "second generation" products to address challenges such as the extreme variability of the HIV virus, which is recognized as an important bottleneck to HIV vaccine development.

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