MOLECULAR MECHANISMS OF POLY [ADP-RIBOSE] POLYMERASE-1 IN HIV-1 INFECTION

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ABSTRACT

Poly *(*ADP-ribose*)* polymerase-1 (PARP-1) is a cellular enzyme involved in genome stability and transcriptional regulation. The role of this protein in HIV-1 infection is largely controversial. Some reports indicated a fundamental role of PARP-1 in HIV-1 DNA integration and results from other laboratories do not support these conclusions. An important characteristic in all these experiments is that the HIV-1 target cells that were used express, in addition to PARP-1, the functional homologue PARP-2. We evaluated the role of PARP-1 in the chicken B lymphoblastoid cell line DT40. These cells naturally lack PARP-2 and support the early steps of HIV infection. We have observed that DT40 PARP-1 -/- cells was significantly more susceptible to infection with HIV- and murine leukemia virus (MLV)-derived viral vectors than their wild type counterpart. Expression of human PARP-1 in DT40 PARP-1 -/- cells decreased retroviral susceptibility to wild type levels, while expression of human PARP-2 has only a partial effect. Analysis of the retroviral life cycle by real time PCR indicated that levels of retroviral provirus were similar in DT40 wild type, PARP-1 -/-, and PARP-1 -/- cells expressing human PARP-1. These results, suggested that retroviral latency was established in cells expressing PARP-1. Treatment of HIV- or MLV-infected cells with the histone deacetylase inhibitor sodium butyrate or the DNA methyl transferase 1 inhibitor 5-azacytidine suppressed the differences in retroviral transgene expression observed in cells expressing or not PARP-1.

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Chapter 1

BACKGROUND AND SIGNIFICANCE

The HIV pandemic. Without a doubt, the Human Immunodeficiency Virus (HIV) pandemic is the most serious infectious disease challenge to public health today. According to the most recent AIDS epidemic updates (2010), 33.3 million people are living with HIV, whereas 2.7 million newly infection and 1.8 million deaths were reported just in this year. This trend in the infection determines that the daily rate of new infections worldwide surpasses 7,000 persons worldwide and the daily mortality rate is over 5,700 persons (15).

Though there has been significant progress in HIV research, treatment and prevention, HIV continued infection and mortality rates outpace that progress, and the disease remains at pandemic levels. Advances in antiretroviral drugs have significantly reduced in AIDS-related deaths, delayed the progression of the HIV infection to AIDS and diminished the rates of HIV transmission (15, 47). However, the current antiretroviral drugs do not target latent infection and therefore cannot eradicate HIV-1 infection. Therefore there is an important need of understanding the HIV latent infection to design drugs that specifically target this condition. This thesis focuses on the characterization of molecular mechanisms of HIV-1 latency.

HIV Life cycle. HIV is a retrovirus that is part of the *Lentivirus* genus, of the retroviridae family. HIV is able to enter the cell through a viral – cell membrane fusion mechanism. Fusion occurs through the interaction of viral glycoproteins that are present in the envelope and a receptor and co-receptors in the host cell membrane. The specific viral glycoproteins involved in this process are gp120 and gp41, and the cellular membrane receptors CD4 and coreceptors CCR5 or