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HIV-1 Latency and AIDS Pathogenesis in Association with p53 and HIV-1 Tat

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Abstract

It has been well known that HIV-1-infected patients show AIDS symptoms generally after long asymptomatic periods. Even though high titer of HIV-1 was detected in the spleen or lymph node of the patients in this period, HIV-1 replication seemed to be strongly suppressed in the body until second viremia. Few papers have reported the HIV-1 inhibition in line with p53 of infected cells. However, the detail mechanism for the p53-medicated HIV-1 suppression has not yet been clearly revealed. We found that the p53 is unlikely to act directly on the HIV-1 LTR or Tat. In the consecutive experiments, we found that the Tat was phosphorylated by transfection of p53 and the phosphorylation intensity was increased in proportional to the amounts of transfected p53. Through the kinase experiment we found that PKR is likely to be involved in the p53-mediated Tat suppression. Recombinant PKR was co-immunoprecipitated with recombinant Tat, when tested with anti-Tat antibody. We found that PKR-Tat interaction was much more stronger than PKR-eIF-2a in yeast two hybrid experiments. The expression of PKR was markedly enhanced by the expression of p53. It means that PKR-Tat interaction depends on the p53 and the interaction seems to result in PKR-mediated Tat-phosphorylation. PKR-knock out cells didn't show the p53-mediated Tat suppression. Four out of 7 mutant Tats having point mutation at ser/thr-residues, respectively, were not inhibited for their transactivation capacity by p53 transfection, indicating that p53-mediated Tat suppression is strongly associated with Tat phosphorylation at a specific sites via activated PKR. It was also newly found that the PKR-mediated Tat-phosphorylation blocks the Tat/TAR binding, followed by inhibition of the Tat-mediated transcription. It means that the HIV-1 latency in the early stage of infection is due to the p53-triggered Tat-phosphorylation through activated PKR. However, in later stage, the amounts of accumulated Tat seem to overcome the p53-triggered PKR-mediated Tat-phosphorylation, resulting in second viremia and full-blown AIDS. PKR-mediated phosphorylation sites on Tat and biological functions of phospho-Tat, investigated, will be presented and discussed.

Rationale and Experimental Results

Since its isolation in 1983, human immunodeficiency virus type 1 (HIV-1) continues to cause 5 million new infections each year, and since the beginning of the epidemic, 31 million people have died as a result of HIV/AIDS. HIV-1 infection causes acquired immunodeficiency syndrome (AIDS) after a long clinical latency. The existence of host suppressors for HIV-1 replication has been discussed because HIV-1 infection has an AIDS latency varying from several months to as long as decades. Wild-type p53 was suggested as a candidate suppressor since it has been shown to suppress viral replication in several experiments. During the infection, human $CD4^+$ T cells have been shown to express p53. p53 induction and activation in the HIV-1 infected cells were confirmed in vitro. Similarly, in patients infected by HIV-1, it has been found that a significant fraction of lymph node cells stained positively with antibodies recognizing p53. Among PBMC, functional p53 was mainly found among $CD4^+$ and $CD14^+$ cells, correlating with the viral load.

Several papers have shown that p53-mediated HIV-1 suppression has to do with viral Tat protein, a major transactivator of HIV-1. The latent infection of HIV-1 was reported in line with p53 and Tat in the infected cells. However, the detail mechanism for the p53-medicated HIV-1 suppression has not yet been clearly revealed. On the other hand, interferon (IFN)-induced, dsRNA-dependent serine/ threonine protein kinase, PKR, plays a key regulatory role in the IFNa-mediated anti-viral response by blocking translation in the infected cell by phosphorylating the alpha subunit of elongation factor 2 (eIF2). As reported in other viral replications, HIV-1 replication was inhibited by IFNa treatment, and PKR was confirmed to be involved in the inhibition mechanism. However, eIF2a-knock down did not block the IFN-mediated HIV-1 inhibition, suggesting that IFN-mediated HIV-1 inhibition is unlikely to be associated with PKR-eIF2a pathway.

Our work was initiated to identify the p53-mediated HIV-1 latency and AIDS pathogenesis in line with the signaling pathway among the p53, PKR and Tat. HIV-1 Tat is a 14 kDa viral protein involved in the regulation of HIV-1 transcriptional elongation and in its presence, viral replication increases by greater than 100-fold. It functions to trigger efficient RNA chain elongation by binding to TAR RNA, which forms the initial portion of the HIV-1 transcript. The interaction between Tat and TAR is critical for virus replication and mutations in Tat that alter the RNA-binding site result in defective viruses. Furthermore, virus replication can be strongly inhibited by the overexpression of TAR RNA sequences that act as competitive inhibitors of regulatory protein binding. While a number of reports have shown that PKR and Tat protein interact, and furthermore, that Tat is phosphorylated by PKR, none have yet addressed the issue of the functional consequences for the phosphorylation of the Tat protein. Here we examine the phosphorylation of Tat by PKR and its effect on TAR RNA binding and HIV-1 transcription. In our experiments, we found that HIV-1 infection induced p53 expression and activation

followed by PKR induction in the transcription level. Enhanced and activated PKR phosphorylates Tat at 5 Ser/Thr sites of Tat protein in allosteric manner starting at Ser-40. PKR-Tat interaction was much stronger than that between PKR-eIF2a. PKR-mediated Tat phosphorylation made Tat lose its TAR-binding capacity and nuclear localization. Phospho-Tat remains mainly in the cytoplasm, which probably results in HIV-1 mediated $CD4^+$ T cell depletion in AIDS pathogenesis.

Conclusion

Overall, these results suggest that the phosphorylation of Tat by PKR plays a key role in the p53-mediated HIV-1 suppression, resulting in the long clinical latency in AIDS pathogenesis. This may, in part, explain the advantage of IFN treatment in patients with early AIDS. The gradual increase of HIV-1 replication in the later stage of AIDS patients is likely associated with the absence of phosphorylation residues on Tat, which are essentially required for the inhibition of Tat-mediated transactivation, resulting from the lack of functional PKR and p53.