HIV/AIDS IS ERADICABLE, IF HIV/AIDS IS CURABLE (Part three)

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Abstract

HIV-1 encodes the regulatory protein Tat (86–101aa), which is essential for HIV-1 replication and primarily orchestrates HIV-1 provirus transcriptional regulation. Tat protein plays a key role in the pathogenesis of both HIV-1-associated cognitivemotor disorder and drug abuse.

Some studies suggest that neurological involvement of infected patient occur with different frequency, depending on HIV subtype involved in the infection. Subtype C may have reduced neuroinvasive capacity, possibly due to its different primary conformation of HIV transactivating regulatory protein (Tat), involved in monocyte chemotaxis.

Previous studies have demonstrated that Tat function is highly dependent on specific interactions with a range of cellular proteins.

The in vitro Tat nuclear interactome and have highlighted its modular network properties and particularly those involved in the coordination of gene expression by Tat.

The ability of Tat to induce morphological changes and promote adhesion was independent of the ability of Tat to transactivate HIV gene expression.

In this article, I discuss the Tat HIV-1 Protein Biological Properties, Biological role of Tat protein in HIV-1 ,Protein Btat versus Ctat

Key Words: Tat, Apoptosis, Mitochondrial, CNS, Cytokine, Chemokine, RNA, In vitro,

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1. Background

Tat protein is a potent transactivator of viral transcription directed by the HIV-l long terminal repeat (LTR). Tat function requires the binding of Tat to a sequence in the LTR known as the transacting response element (TAR) (1).Tat has been demonstrated to affect both transcription initiation and elongation (2). In addition, Tat has been shown to induce morphological changes in certain cell types, enhance cell growth, and promote adhesion.

The molecular mechanisms whereby HIV-1 gene expression is regulated by Tat occurs at distinct levels. Initially, Tat enhances transcription initiation by promoting the assembly of the RNA polII complex by interacting with various transcription factors (3). Subsequently, Tat activates elongation via two independent mechanisms: firstly, it enhances the processivity of RNA polII by interacting with elongation factors such as pTEF-b, which phosphorylates RNA polII C-terminal domain, and secondly, by recruiting histone acetyl-transferase proteins which modify the chromatin template such as p300/CBP (CREB binding protein) and p300/CBP-associated factor (PCAF) and, as recently described, by interacting with BRM and BRG1, two chromatin remodelers (4),(5),(6),(7),(8),(9).

Tat is a small and compact protein, composed of only 86 or 101 amino acids, sequence and functional analysis reveals that Tat sequence encompasses a unique arrangement of five distinct and contiguous regions including the acidic, cysteine-rich, core, basic and glutamine-rich regions. Furthermore, Tat is subject to post-translational modifications, such as acetylation, methylation, phosphorylation and ubiquitination, thus increasing both the number and diversity of potential interfaces between Tat and cellular proteins (10),(11),(12).

Recently, a structural study employing nuclear magnetic resonance (NMR) spectroscopy has described Tat as a "natively unfolded" protein with fast dynamics lacking a well-structured three-dimensional fold. These characteristics would provide Tat the flexibility to interact with numerous cellular partners. Collectively these findings suggest that Tat is a potent, versatile protein suited for multiple interactions and highlights the concept that numerous protein-protein interactions underlie the molecular mechanisms of HIV-1 molecular pathogenesis (13),(14), (15),(16),(17). In this article Protein Biological Properties, Biological function, immunotherapy and modulatory

2. Tat HIV-1 Protein Biological Properties

The HIV-1 promoter is located in the 50 LTR and contains a number of regulatory elements important for RNA polymerase II transcription. Sites for several cellular transcription factors are located upstream of the start site, including sites for NF- ·B, Sp1, and TBP (2).

These cellular factors help control the rate of transcription initiation from the integrated provirus, and their abundance in different cell types or at different times likely determines whether a provirus is quiescent or actively replicating. Under some conditions, Tat may also enhance the rate of transcription initiation (2).

Tat increases production of viral mRNAs »100-fold and consequently is essential for viral replication. In the absence of Tat, polymerases generally do not transcribe beyond a few hundred nucleotides, though they do not appear to terminate at specific sites (18).

Tat binds not to a DNA site but rather to an RNA hairpin known as TAR (trans-activating response element), located at the 50 end of the nascent viral transcripts. An arginine-rich domain of Tat helps mediate binding to a three-nucleotide bulge region of TAR, with one arginine residue being primarily responsible for recognition (19),(20).

TheTat proteinis typically produced by the HIV virus as soon as it enters the human body inorder to ensure the sustainability of the virus, It is involved in viral transcription (21),(22), helping the virus to spread by penetrating the host cell membrane (23),(24). This process begins once the virus enters the human body.

The Tat protein is present from the early stage of HIV infection. Tat cantravelin and out of the cell without being detected through passive diffusion (25). It binds to phospholipids and travels freely across the nuclear membrane (26). The presence of Tat protein in the extracellular environment is toxic and plays a key role in the progression from HIV to AIDS (27).

Tat is released by HIV-infected cells and is detectable in infected human blood serum down to levelsof 40ng/ml (26),(28). The Tat protein can be divided into five different regions according to its aminoacid sequence; aminoacids1–19 (N-terminal activation region),amino acids 20–39 (cysteine-richdomain), amino acids 40–47(coreregion), aminoacids 48–56 (basicregion) and aminoacids57–71 (glutamine-richregion) (29),(30).

The cysteine-rich region is believed to be involved in metal-ion binding(31),(26), while the core, basic and glutamine-rich regions are all involved in RNA binding, which will ater be used as the region for Tat protein detection (32)

Virotoxins are able to increase their potential toxicity by interacting with non-infected cells, damaging non-adjacent sites(33). One of the main proteins related to HIV associated neuronal damage is tat. Its activity is necessary to viral replication(33).

Tat is the only protein actively secreted by HIV-1 infected cells by means of an energy dependent process. It circulates in the blood at high levels during HIV infection and can cross BBB, and large quantities of this protein enter the CNS.(33).

Tat protein easily penetrates different cellular types and contributes to HIV transactivation in infected cells.(34)

As soon as tat is secreted by infected cells, it is picked up by cells causing cytoplasmic and nuclear events, like alteration in gene transcription, cytokine and chemokine secretion, receptor activation and expression of apoptotic protein(27).

Tat is internalized by neurons by to low-density lipoprotein recepto-related protein (LRP) receptor expressed on cellular surface. For the occurrence of neuronal apoptosis, tat must bin to LRP and N-methyl-D-aspartate (NMDAR) receptor. Apoptosis cascade starts in neurons that express these receptors and spread to neurons that do not express these receptors and to astrocytes.(27).

Besides apoptosis, mitochondrial disorders, cytochrome c release, calcium excess and caspase 3 cascade activation also occur in neurons. Extracellular tat induces depolarization and intracellular calcium elevation in neurons, which could be responsible for neuronal death (35).

The increase in cytoplasmic calcium level leads to the production of several oxygen reactive species, including super oxide, hydrogen peroxide nitric oxide and peroxynitrite, molecules that may damage mitochondrial membrane and DNA (35).

3. The Biological role of Tat protein in HIV-1

Tat is the only protein actively secreted by HIV-1 infected cells by means of an energy dependent process. It circulates in the blood at high levels during HIV infection and can cross BBB, and large quantities of this protein enter the CNS. Tat can depolarize neurons through direct interaction with the membranes of these cells, can act like substrate to adhesion and induce aggregation of neural cultures (34, 36).

Tat protein easily penetrates different cellular types and contributes to HIV transactivation in infected cells. It specifically interacts with the vascular endothelial growth factor (VEGF) and its connection to these surface molecules activates several protein kinases. Protein kinase C is a signal molecule that mediates tat effects in microvascular endothelium, which impacts endothelial cell proliferation and migration (34).

As soon as tat is secreted by infected cells, it is picked up by cells causing cytoplasmic and nuclear events, like alteration in gene transcription, cytokine and chemokine secretion, receptor activation and expression of apoptotic protein (27).

Tat has a strong monocyte chemotactic property. It acts like a chemokine itself and/or indirectly through monocyte chemotactic protein 1 (MCP-1) currently classified as CCL2- secreted by astrocytes, the most numerous cells in the central nervous system and that are in close contact with BBB (37).

A little amount of infiltrate monocytes can trigger a cascade of events, leading to immune activation, oxidative stress, decrease in intracellular glutathione (responsible for redox status

maintenance and antioxidant protection of endothelial cells), barrier breakdown and great monocyte influx that together can result in neurological damage progression (38),(35),(39).

Tat is internalized by neurons by to low-density lipoprotein recepto-related protein (LRP) receptor expressed on cellular surface. For the occurrence of neuronal apoptosis, tat must bind to LRP and N-methyl-D-aspartate (NMDAR) receptor. Apoptosis cascade starts in neurons that express these receptors and spread to neurons that do not express these receptors and to astrocytes. NMDAR subunits are phosphorylated when tat potentiates glutamate excitotoxicity in a protein kinase C and tyrosine kinase dependent manner (27).

Besides apoptosis, mitochondrial disorders, cytochrome c release, calcium excess and caspase 3 cascade activation also occur in neurons. Extracellular tat induces depolarization and intracellular calcium elevation in neurons, which could be responsible for neuronal death (35).

Two alterations that apparently contribute to the apoptosis process are: increase in oxygen reactive species production and break of calcium homeostasis. The increase in cytoplasmic calcium level leads to the production of several oxygen reactive species, including super oxide, hydrogen peroxide nitric oxide and peroxynitrite, molecules that may damage mitochondrial membrane and DNA (35).

Tat induces TNF-α release by macrophages and it has an important role in HIV infection pathogeneses. TNF-α is a mediator of CNS inflammatory events. It activates microglia cells, monocytes and macrophages. It is a potent inductor of inflammatory response and can stimulate an increase in CCL2, inflammatory cytokines, ICAM-1, VCAM-1 expression. Also, it can act like a chemoattractant factor, though its chemotactic response is smaller than CCL2 (39).

Nuclear factor (NF-κB) is a transcriptional factor that activates many viral and cellular promoters and has binding places to cytokine promoters. Moreover, it regulates adhesion molecules, chemokine and cytokine expression. TNF-α induces NF-κB translocation to the nucleus increasing inflammatory cytokine expression in astrocytes. NF-κB may influence CCL2 expression directly. This bind is generally associated with protein kinase activation (40).

Tat chronic production in brain causes significant alteration in inflammation histological markers and in the infected individual's behavior (41).

4. Protein Btat versus Ctat

Non-C subtypes protein tat are conserved to preserve a dicysteine-containing motif (30 and 31 positions), necessary to monocyte migration. HIV subtype C shows a replacement of cysteine with serine, thus breaking dicysteine motif and reducing monocyte attraction ability. More than 99% of non C subtypes present cysteine in 31 position and around 90% of subtype C sequences present a serine in this position (37).

Some differences between subtype B tat and subtype C-tat are observed. B-tat caused greater neuronal apoptosis compared to subtype C. C-tat caused lower oxidative stress in neurons. B-tat is more flexible than C-tat, which can explain its greater biological activity (42).

When CCL2 binds to CCR2, an increase in arachidonic acid release, extracellular calcium influx and chemotaxis occurs. A mutation in CCF sequence to SSG annuls the ability to induct calcium influx, resulting in the failure of tat protein binding to CCR2, decrease in chemotactic activity and in the ability to induce inflammatory mediators, like IL-6, CCL2 and TNF-α (43).

5. Conclusion and Remarks

Lentivirus-based methods of gene delivery have proven their indisputable superiority over simple retroviruses in safe modification of quiescent, non-stimulated hematopoietic progenitor cells *ex vivo*, potent delivery of genetic payload to neurons in the CNS and to other differentiated cells in distinct organs. HIV-1 Tat rewiring of cellular networks could equip the provirus with a wide repertoire of tools to orchestrate HIV-1 gene expression and confer a remarkable adaptability to a continuously changing cellular environment.

Tat transactivation function appears to be the net result of complex interactions with distinct cellular complexes highly specialized in controlling gene expression and more specifically chromosome/chromatin structure.

Tat-mediated aggregation and attachment of neurons required the cysteine-rich basic and RGD domains of Tat. The RGD motifpresent in ECM proteins such as fibronectin and vitronectin mediates the interaction of these proteins with their cognate integrins (44).).

Tat interacts directly with integrins (45). Because mutation of the RGD sequence or antibodies against the RGD motif eliminated the ability of Tat to promote aggregation and adhesion of cerebellar neurons, our data further demonstrate the involvement of the RGD sequence in mediating the effects of Tat on cell adhesion and morphology. Tat also mediated aggregation and adhesion of neurons.

Our data also support a role for the basic domain of Tat in mediating the effects of Tat on cerebellar neurons. Interestingly, deletion of the cysteine-rich region eliminated the ability of Tat to cause neuronal
aggregation and adhesion.

Several studies have demonstrated that Tat promotes morphological changes in cells. Mice transgenic for HIV-l Tat have been shown to develop KS-like lesions (46).

Tat and fibronectin synergize with basic fibroblast growth factor to cause KS-like lesions in normal vascular endothelial cells, further suggesting that Tat interacts with integrins (47).

The ability of Tat to cause aggregation diminished as the cultures matured, suggesting that Tat interfered with the ability of neurons to make productive cell-cell and cell-substrate connections.

The neurotoxicity of Tat peptides appears to be nonspecific, because peptides comprised of the basic domain of sheep visna virus Tat and HIV-l rev, which possess considerable homology to the Tat basic domain, were also reported to be neurotoxic (48).

Extracellular Tat has been shown to transactivate the HIV-l LTR in neural cells (49), and activates expression of certain cellular genes in a TARindependent manner. Expression of TNF-a and TNF-/3 has been shown to be increased by Tat in lymphoid cells (50). In astrocytes, TAR-independent transactivation by Tat is mediated by NF-KB sequences in the HIV-l LTR (51),(52). TAR-independent transactivation by Tat did not require the presence of the basic domain but did require the amino-terminal "activation" domain of Tat (53).

The pathological effects of HIV infection in the brain likely result from indirect effects of HIV gene products and cytokines released by other cells within the CNS that are more permissive for HIV infection (54). Monocytes, macrophages, glial cells, and astrocytes are the major targets of HIV-l infection in the brain (55),(56),(57),(58). Cytokines released by HIV-infected cells in the brain (59),(60), have been shown to be neurotoxic both alone and in combination with cell-to-cell interactions (61),(62),(63),(64). Cytokines also have been reported to upregulate HIV gene expression in various cell types found in the brain (65),(66),(67). which may increase expression of Tat.

It is possible that the effects of Tat on cerebellar and cortical neurons may be mediated by a similar mechanism. Tat-mediated adhesion and aggregation of neurons and whether Tat directly or indirectly affects cytokine expression. Further studies will hopefully lead to a better understanding of the roles of Tat and cellular factors in the pathogenesis of HIV infection in the brain and biological significance of these findings

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