

Review

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Functions of Tat: the versatile protein of human immunodeficiency virus type 1

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Human immunodeficiency virus type 1 (HIV-1) Tat is a multifunctional protein that contributes to several pathological symptoms of HIV-1 infection as well as playing a critical role in virus replication. Tat is a robust transactivating protein that induces a variety of effects by altering the expression levels of cellular and viral genes. The functions of Tat are therefore primarily related to its role in modulation of gene expression. In this review the functions of HIV-1 Tat that have been well documented, as well as a number of novel functions that have been proposed for this protein, are discussed. Since some of the functions of Tat vary in different cell types in a concentration-dependent manner and because Tat sometimes exerts the same activity through different pathways, study of this protein has at times yielded conflicting and controversial results. Due to its pivotal role in viral replication and in disease pathogenesis, Tat and the cellular pathways targeted by Tat are potential targets for new anti-HIV drugs.

Introduction

Normal cell functioning of viral hosts is altered by invading virus proteins to the benefit of the virus (Evans *et al.*, 2009). Viral proteins are known to compete with the host proteins thus disrupting the normal host protein–protein interaction network (Sodhi *et al.*, 2004; Tournier & Quesnel-Hellmann, 2006). Molecular studies have revealed nearly 1500 interactions between human immunodeficiency virus type 1 (HIV-1) and human proteins, these interactions are catalogued in the HIV-1, Human Protein Interaction Database (Fu *et al.*, 2009; Pinney *et al.*, 2009; Ptak *et al.*, 2008). Efforts to understand the interactions between viral and cellular gene products that together determine the host's susceptibility to infection and disease have led to significant new insights about HIV-1 (Samuel, 2006).

In addition to the structural genes (*gag*, *pol* and *env*), the HIV-1 genome contains four accessory (*vif*, *vpr*, *vpu* and *nef*) and two regulatory (*tat* and *rev*) genes, the products of which are responsible for establishing sophisticated interactions between the virus and human host (da Silva *et al.*, 2006; Zuo *et al.*, 2006). HIV-1 Tat is well-known as a transactivator protein that contributes to transactivation of viral and cellular genes (Ju *et al.*, 2009; Mahlknecht *et al.*, 2008; Nekhai *et al.*, 2007). Tat is an early regulatory protein that has a variable length of 86–104 aa, encoded by two exons (Fig. 1). The first exon encodes the first 72 aa (Pugliese *et al.*, 2005; Schwarze *et al.*, 1999). Due to the variable length of Tat, its weight varies from 14 to 16 kDa. Incomplete forms of this viral protein (from 58 to 72 aa)

may also be able to induce the biological affects of the full-length protein. A double splicing mechanism occurs after the transcription of *tat* mRNA. This is a post-transcriptional modification that consists of cutting of the *tat* mRNA and removal of unnecessary sequences. The process is followed by the joining together of nucleic acid sequences (Fanales-Belasio *et al.*, 2009; Pugliese *et al.*, 2005).

The extracellular form of Tat, which is released from productively infected cells, is also able to enter target cells and induce its effects (Ferrari *et al.*, 2003; Zheng *et al.*, 2005). Studies of Tat-derived peptides have demonstrated that residues 48–60 from the basic domain (the protein transduction domain or PTD) account for the functional internalization into cells (Futaki *et al.*, 2001; Schwarze *et al.*, 2000) and that cellular heparan sulfate proteoglycans act as low-affinity cellular receptors for extracellular Tat (Tyagi *et al.*, 2001). Mutational analysis of HIV-1 Tat has identified two important functional domains: an activation domain that mediates its interactions with cellular machinery and an arginine-rich region that is required for binding to the transactivation responsive element (TAR) RNA (Hwang *et al.*, 2003).

Role of Tat in synthesis of full-length viral transcripts

Functions proposed for HIV-1 Tat include chromatin remodelling, phosphorylation of RNA polymerase II that is involved in the transcription of the full-length viral mRNAs, transactivation of viral genes and binding to a

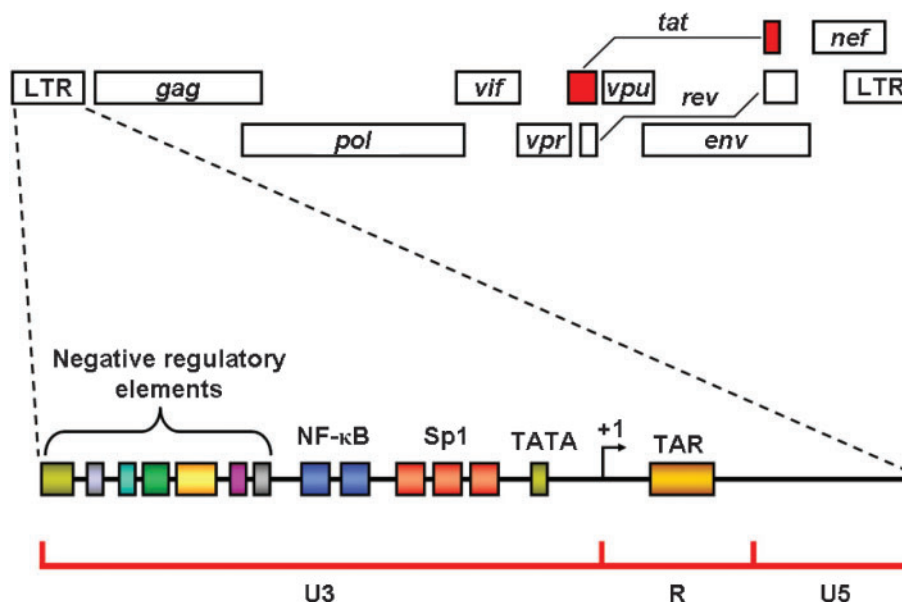


Fig. 1. Structure of the HIV-1 genome. Tat is encoded by two exons (in red). The first exon codes for the first 72 aa, which are sufficient for Tat transactivation. The second exon codes for amino acids 73–104. The detailed structure of the LTR is shown at the bottom of the picture. The HIV-1 LTR contains many protein-binding sites. Some of the protein-binding sites, such as negative regulatory elements, downregulate transcription of the HIV-1 genome through binding to regulatory proteins. The HIV-1 LTR also contains a TATA box, two direct repeats of NF- κ B-binding site and three tandem repeats of Sp1-binding site. Tat recruits transcription factors on the LTR to upregulate the transcription of the HIV-1 genome.

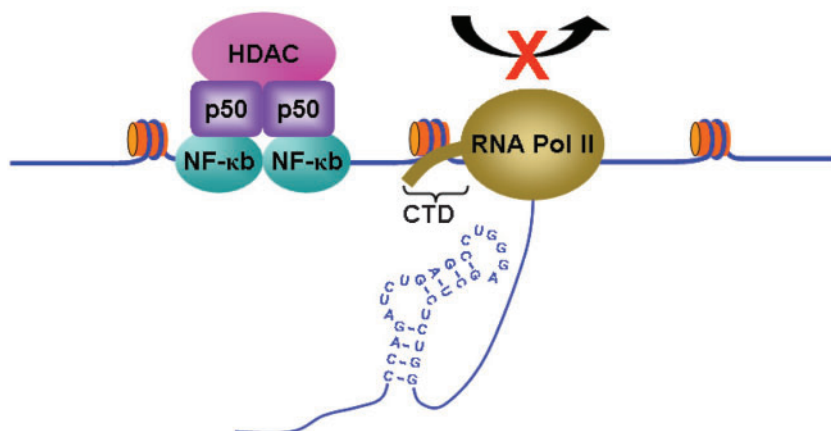
specific structure of HIV-1 mRNAs (Ammosova *et al.*, 2006; Richman *et al.*, 2009; Wong *et al.*, 2005; Yedavalli *et al.*, 2003). A glance at these functions may suggest separate functions for one single protein. However, the thorough study of Tat suggests all of these functions are sequentially triggered as a cascade for a single purpose, namely, HIV-1 gene expression. In fact, HIV expression is limited by cellular barriers which inhibit effective mRNA transcription. The HIV-1 provirus overcomes these barriers through the action of its own activator, Tat (Pumfery *et al.*, 2003). Tat recruits cellular proteins to relieve the repression of the viral long-terminal repeat (LTR), and thereby the viral promoter can induce the expression of viral genes (Richman *et al.*, 2009).

In the absence of any stimulation, the integrated HIV-1 provirus remains silent in latently infected cells such as T cells, monocytes and macrophages. Expression of the viral genome is regulated by the enhancer and promoter elements contained within the HIV LTR located at the 5' end of the integrated HIV provirus (Fig. 1) (Rohr *et al.*, 2003). HIV-1 Tat interacts with several chromatin modifying complexes and histone modifying enzymes to relieve the virus LTR. Tat recruits various histone acetyltransferases (HATs), such as the CREB-binding protein (CBP)/p300 complex, to the HIV-1 promoter region, and thereby the nucleosomes on the promoter are acetylated by these enzymes (Fig. 2). Tat-induced acetylation of the nucleosomes by CBP/p300 relieves the

repression of the LTR promoter such that the transcription by RNA polymerase II is not aborted and the enzyme efficiently transcribes the viral genes (Deng *et al.*, 2000; Pumfery *et al.*, 2003). *In vitro* studies have demonstrated that Tat changes the conformation of the CBP/p300 complex such that it can bind better to basal transcription factors such as the TATA-binding protein and the transcription factor IIB (Deng *et al.*, 2000, 2001). It has also been shown that Lys-28 in the activation domain of Tat, which is essential for HIV-1 transcription and replication, is acetylated by the p300/CBP-associated factor (PCAF) (D'Orso & Frankel, 2009). Triggering the transcription factors suggests that Tat may influence the transcription machinery by helping the CBP/p300 complex to recruit new partners into the transcription machinery (Deng *et al.*, 2000).

In the prevailing model of HIV transcription, the RNA polymerase II complex is constitutively bound to the HIV promoter and persistently initiates the synthesis of short mRNAs (Williams *et al.*, 2006). However, in the absence of Tat or cellular activation signals, the efficiency of the bound RNA polymerase is dramatically reduced, resulting in the production of abortive short viral transcripts (Fig. 2a) (Pagans *et al.*, 2005). Tat binds to an RNA stem-loop structure, called TAR, which is located at the 5' end of all initiated viral transcripts. Tat also recruits P-TEFb complex which is composed of cellular cyclin T1 and CDK9 proteins (Zhou *et al.*, 2003). The P-TEFb complex

(a)



(b)

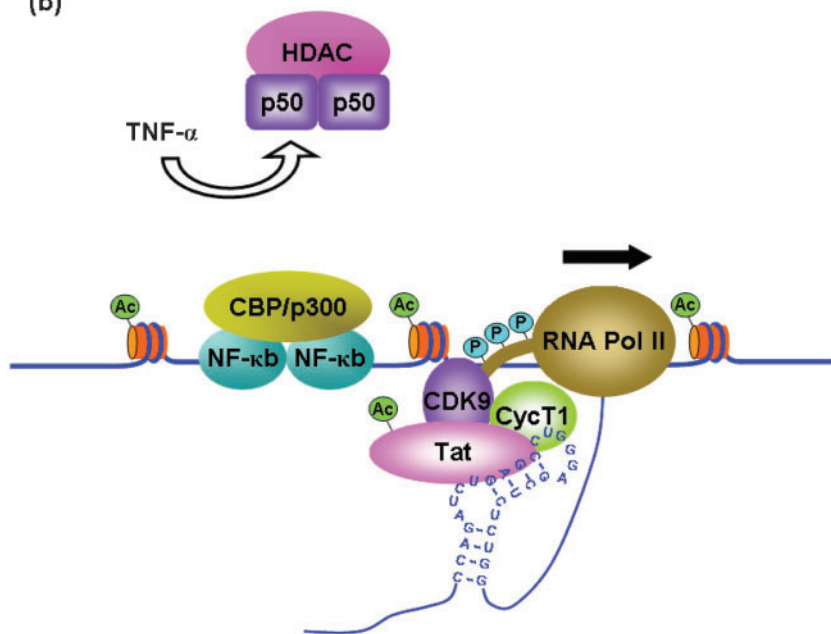


Fig. 2. The role of HIV-1 Tat in the synthesis of full-length viral mRNAs. (a) NF- κ B, p50 and HDAC1 complexes bind to the latent HIV-1 LTR and induce histone deacetylation and repressive changes in chromatin structure of the HIV-1 LTR. In the absence of HIV-1 Tat, although RNA polymerase II initiates synthesis of the virus transcripts, it fails to complete the synthesis of full-length transcripts. (b) In the presence of stimulation, such as TNF- α , the complexes inducing latency, p50 and HDAC1, are removed from the viral LTR. The CBP/p300 complex binds to the promoter and acetylates the surrounding histones. The complex also mediates acetylation of Tat using PCAF (not shown). Tat interacts with the P-TEFb complex, composed of CDK9 and cyclin T1, to recruit the stem-loop structure, TAR, which acts as a promoter element in the transcribed 5' end of the viral LTR. The P-TEFb complex activates RNA polymerase II by phosphorylating the polymerase in CTD. The activated polymerase can successfully synthesize the full-length transcripts from the relieved template.

phosphorylates serine residues in the C-terminal domain (CTD) of RNA polymerase II. These events potentiate RNA polymerase II to synthesize full-length HIV transcripts (Williams *et al.*, 2006). Tat-induced serine phosphorylation of CTD by P-TEFb also stimulates the human capping enzyme, leading to capping of HIV-1 mRNAs (Zhou *et al.*, 2003).

Besides the RNA polymerase II complex, the phosphorylation of additional transcription factors, including Sp1, CREB, the α subunit of eukaryotic initiation factor 2 (eIF2 α) and NF- κ B have also been reported to be triggered by Tat (Demarchi *et al.*, 1999; Li *et al.*, 2005; Rossi *et al.*, 2006; Zauli *et al.*, 2001). Tat has been indicated to influence the HIV-1 promoter by phosphorylation of Sp1. Three

tandem repeats of Sp1-binding sites have been identified in HIV-1 LTR (Fig. 1). Sp1 plays a critical role in many cellular events by regulating the expression of cellular genes. Tat activates Sp1 by phosphorylation of this protein, and thereby Sp1 proteins bind to LTR to upregulate the HIV-1 promoter (Chang *et al.*, 1994; Rossi *et al.*, 2006). The interaction of Tat with the HIV-1 accessory protein Vpr has also been shown to enhance the transcriptional activity of the HIV-1 LTR. Co-transfection studies using HIV-1 LTR luciferase reporter plasmid and Tat-plus-Vpr-expression vector indicate superactivation of LTR by Tat and cyclin T1/CDK9 in the presence of wild-type Vpr. Results from protein-protein interaction studies have demonstrated that Vpr is associated with both Tat and cyclin T1 in cells expressing these proteins (Sawaya *et al.*, 2000).

Tat-induced modulation of cellular gene expression

In addition to the regulatory roles of HIV-1 Tat in the transactivation of virus genes, Tat has been implicated as a modulator of expression levels of a number of cellular genes (Li *et al.*, 2005; Stettner *et al.*, 2009). For example, Tat has been shown to upregulate the expression levels of a number of cytokines, the HIV-1 co-receptor CCR5 and the interleukin-2 (IL-2) receptor (CD25) in HIV-1-infected cells (Mayol *et al.*, 2007; Stettner *et al.*, 2009; Zheng *et al.*, 2005). Tat also downregulates several genes, such as the gene encoding major histocompatibility complex (MHC) class I (Matsui *et al.*, 1996). The transactivating effects of Tat are not limited to HIV-1-infected cells because the extracellular Tat protein, released from virus-infected cells, is able to enter uninfected cells and exert its activity directly on responsive genes (Ju *et al.*, 2009). The upregulation of cellular genes by Tat consequently induces additional effects, such as apoptosis induction and immunosuppression (Gupta *et al.*, 2008; Poggi & Zocchi, 2006; Bannasser & Bahraoui, 2002; Zheng *et al.*, 2005), which will be discussed later.

In vivo studies have shown that Tat deregulates the expression levels of several cytokines (Scala *et al.*, 1994; Westendorp *et al.*, 1994; Bannasser & Bahraoui, 2002). By using a vector expressing IL-6 under the control of the IL-6 promoter, it has been demonstrated that Tat transactivates the human IL-6 promoter (Scala *et al.*, 1994). Ambrosino *et al.* (1997) have identified a short sequence at the 5' end of the IL-6 mRNA that is required for Tat to transactivate the IL-6 promoter. This sequence acquires a stem-loop structure and contains a UCU sequence that binds to Tat and is necessary for full transcription of IL-6 mRNA. Li *et al.* (2005) have examined the mechanism involved in Tat-induced cytokine dysregulation and found that Tat upregulates cytokine expression through protein kinase R (PKR) activation in primary blood monocytes. A recent study has demonstrated that subtype B and subtype C Tat protein exert differential cytokine modulating effects. The study showed that the expression of anti-inflammatory molecules including IL-4 and IL-10 is higher in Tat C-treated compared with Tat B-treated cultures of primary human monocytes. Conversely, subtype B Tat protein shows significant upregulation of pro-inflammatory cytokines, IL-6 and TNF- α , as compared with subtype C Tat protein (Gandhi *et al.*, 2009).

Matrix metalloproteinases (MMPs) can mediate the degradation of extracellular matrix proteins and also degrade components of the blood-brain barrier. The activity of MMPs is strictly regulated at the levels of gene expression. The dysregulation of MMP activity in HIV-infected individuals is associated with disease progression by influencing the integrity of the extracellular matrix. Recently, it has been shown that extracellular Tat upregulates the expression of MMP 9 via an NF- κ B-dependent pathway in astrocytes (Ju *et al.*, 2009).

In addition to Tat protein, transfected TAR RNA has also been shown to attenuate the RNAi machinery in human cells (Bennasser *et al.*, 2006). Recently, TAR miRNA has been implicated in the modulation of cellular gene expression. This miRNA is functional and may be involved in the regulation of the viral life cycle through suppression of viral transcription. The TAR mRNA is expressed at all stages of the viral life cycle and can be detected in latently infected cells. An *in vitro* study using TAR miRNA expression vector has shown that TAR miRNA protects infected cells from apoptosis and acts by downregulating cellular genes involved in apoptosis. The study also shows that the miRNA downregulates excision repair cross complementing group 1 (ERCC1) and intermediate-early response 3 (IER3), protecting the cell from apoptosis (Klase *et al.*, 2009; Ouellet *et al.*, 2008).

Tat-induced modulation of genes from non-HIV viruses

Tat is a robust transactivating protein whose transactivating activity is not restricted to HIV-1 genes alone. In addition to the modulation of HIV-1 genes, Tat has been reported to upregulate a number of non-HIV viral genes, resulting in virus activation (Gorrill *et al.*, 2006; Stettner *et al.*, 2009). In a number of carcinogenic viruses, such as human papillomavirus (HPV) and KS-associated herpesvirus (KSHV) this activation could result in neoplasia (Aoki & Tosato, 2004; Turner & Palefsky, 1998), this is discussed below.

Tat has been implicated in the stimulation of JC virus (JCV) which is the aetiological agent of progressive multifocal leukoencephalopathy. The virus causes demyelinating disease of the brain in HIV-infected individuals. Although HIV-1-induced immunosuppression is involved in infection of the brain by JCV, a direct association with HIV-1 Tat has also been established. Tat has been shown to activate the cytokine transforming growth factor (TGF)- β in HIV-1-infected cells and to stimulate JCV gene transcription and DNA replication in oligodendrocytes, myelin producing cells of the central nervous system (CNS). *In vitro* studies have indicated that Tat stimulates transcription from both early and late gene promoters of JCV (Stettner *et al.*, 2009).

Tat has been demonstrated to stimulate polyomavirus BK (BKV) (Gorrill *et al.*, 2006), which rarely causes disease. Many people who are infected with BKV are asymptomatic. However, the virus is a serious problem for immunocompromised patients, such as HIV patients (Miller *et al.*, 2009). *In vitro* experiments have shown that Tat activates BKV by inducing binding of NF- κ B to a κ B motif within the early promoter of the virus (BKV_E). A sequence within the 5'-untranslated region of BKV_E transcripts (BKV_E-TAR) has been identified that is identical to the HIV-1 TAR. Interestingly, Tat has been shown to bind to BKV_E-TAR to stimulate transcription of the viral genes. *In vitro*

results suggest that Tat stimulates BKV_E transcription by a dual mechanism (Gorrill *et al.*, 2006).

Role of Tat in HIV-1 reverse transcription

Although Tat has not been shown to be incorporated into HIV-1 virions, surprisingly HIV-1 variants carrying mutated *tat* genes fail to efficiently reverse transcribe their genomic RNA. Biochemical analysis of these viruses revealed wild-type levels of each of reverse transcriptase (RT), viral genomic RNA, and Gag and Env proteins (Harrich *et al.*, 1997). However, the viral RNA of the Tat-mutated viruses appeared as partially degraded molecules on agarose gels and not in dimeric form as found within wild-type virions. It has been reported that the conformation of virion RNA is critical for reverse transcription, particularly for the first-strand transfer. It is believed that the degraded nature of the RNA in Tat-mutated virions accounts, at least in part, for the failure of this RNA to undergo efficient reverse transcription in infected cells. It is also possible that the low levels of Tat incorporation into HIV-1 virions may not be detected by currently available techniques. It has been suggested that as few as two molecules of Tat may be incorporated into virions by binding to the two copies of HIV-1 genomic RNA. Hence, Tat can conceivably be associated with the reverse transcription complex and directly participate in reverse transcription following infection of target cells (Bres *et al.*, 2002; Liang & Wainberg, 2002).

In vitro studies have shown that wild-type Tat can increase viral infectivity by suppressing the RT at late stages of the viral life cycle. Tat has been suggested to inhibit reverse transcription in order to prevent the premature synthesis of potentially deleterious viral DNA products. Tat has also been reported to possess an RNA-annealing activity which enables it to promote the placement of tRNA onto viral RNA. The viral nucleocapsid (NC) protein has also been documented to promote the annealing of tRNA^{Lys} as a primer for reverse transcription. The RNA-annealing activity of Tat suggests a functional homology between Tat and NC. Tat appears to play at least two different roles in viral reverse transcription: suppression of DNA polymerization and placement of tRNA onto viral RNA. The first activity may contribute to the efficiency of reverse transcription of the viral genome as well as to the enhanced production of infectious viral particles. The second activity may play a potential role for NC in the displacement of Tat during viral maturation (Kameoka *et al.*, 2002).

Immune suppression by Tat

Immune suppression by Tat is related closely to the effect of Tat on cellular gene expression. Because modulation of gene expression by Tat is not always associated with immune suppression and the suppressive affect of Tat on gene expression could be a consequence of modulation of gene expression alone, the Tat-induced immune suppres-

sion is discussed as an independent function in this paper. IL-10 represents a good example of the relationship between modulation of gene expression and immune suppression by Tat. As already discussed, Tat has been shown to upregulate cytokine production and IL-10 is one of these cytokines. Since IL-10 has significant T cell inhibitory activity, its upregulation by Tat could suppress T cells. Gupta *et al.* (2008) have shown that Tat suppresses gp120-specific T-cell response in an IL-10-dependent manner and the immunosuppressive effect of Tat is not observed in IL-10-deficient mice. Although they have suggested that the immunosuppressive effect of Tat is mediated through IL-10, it seems that Tat may adopt several strategies to suppress the immune system of HIV-infected individuals (Barton *et al.*, 1996; Gallo, 1999; Miura *et al.*, 2003; Poggi & Zocchi, 2006; Zheng *et al.*, 2005).

In vitro studies have demonstrated that Tat could inhibit interferon-induced nitric oxide synthase gene activity in macrophages. The inhibitory effect of Tat on the production of nitric oxide by host macrophages renders the host vulnerable to infections, since nitric oxide provides the first line of defence against opportunistic pathogens (Barton *et al.*, 1996). Tat also activates alpha interferon (IFN- α) production in macrophages. A high level of IFN- α production, which is mediated by Tat, may be involved in induction of immune suppressive effects since overproduction of IFN- α could result in anti-proliferative effects (Gallo, 1999). Although IFNs are known as antiviral agents, Zaguri *et al.* (1998) have shown the involvement of overexpression of IFN- α in the immunosuppression of uninfected T cells. The inhibitory effects of Tat on MHC I expression also suggest another mechanism for induction of immune suppression by Tat. Since MHC I presents intracellular antigens to CD8⁺ T cells, downregulation of MHC I could seriously affect CD8⁺ T cell-associated immune activation and immune responsiveness in general (Dalakas, 2006; Matsui *et al.*, 1996; Mayol *et al.*, 2007).

Tat-induced apoptosis may also be involved in the immunosuppressive activities of Tat. The upregulation of TGF- β transcription by Tat has been claimed to contribute to immunosuppression in AIDS (Poggi & Zocchi, 2006). TGF- β has been reported to induce apoptosis in several cell types through AP-1-dependent activation of the src homology 2 (SH2)-domain containing inositol-5-phosphatase (Licona-Limón & Soldevila, 2007; Tarasenko *et al.*, 2007). The upregulation of TGF- β by Tat reduces anti-apoptotic proteins and thereby renders immune cells vulnerable to apoptosis. The outcome of this event could be the induction of immunosuppression (Poggi & Zocchi, 2006).

Apoptosis induction by Tat

Apoptosis, in general, is regulated through one of two known cell death-signalling pathways: the extrinsic (or death receptor) pathway and the intrinsic pathway. The

extrinsic pathway is triggered by external stimuli or ligands, sensed by cell-death receptors such as Fas/CD95 expressed on the cell membrane. Mitochondria on the other hand play the pivotal role in the intrinsic pathway by releasing apoptosis-inducing molecules, mainly cytochrome *c*, followed by activation of caspases-9 and -3, respectively (Paquette *et al.*, 2005; Ruwanpura *et al.*, 2008). *In vitro* studies have shown that HIV-1 Tat is able to induce the intrinsic pathway of apoptosis in a number of human cell lines. The results of these studies suggest that Tat promotes apoptosis during HIV-1 infection (Giacca, 2005; Kim *et al.*, 2003; Park *et al.*, 2001) although other proteins of HIV-1 (Vpr and gp120) have also been shown to induce apoptosis (Ramalingam *et al.*, 2008; Romani & Engelbrecht, 2009; Vashistha *et al.*, 2009). Extracellular Tat, produced by adjacent infected macrophages, microglia or astrocytes, can interact with the low density lipoprotein receptor-related protein (LRP) on the surfaces of neurons to enter cells by endocytosis. Extracellular Tat has been shown to induce neuronal death by binding to the LRP. Accumulating evidence suggests that extracellular Tat plays an important role in neuronal apoptosis induction, which is associated with the neurotoxic effects of Tat (Kim *et al.*, 2008; Wong *et al.*, 2005).

A link between microtubule polymerization and the pro-apoptotic effect of Tat has been suggested since the interaction of Tat with both non-polymerized subunits of microtubules ($\alpha\beta$ -tubulin dimers) and polymerized microtubules, leads to mitochondrial induction of apoptosis (Fig. 3) (de Mareuil *et al.*, 2005; Giacca, 2005; Vendeville *et al.*, 2004). The functional consequence of this interaction is microtubule stabilization and the consequent prevention of microtubule depolymerization. Since cells are dynamic units requiring constant polymerization and depolymer-

ization of microtubules, this change in microtubule stabilization can act as a powerful inducer of the intrinsic pathway of cellular apoptosis (Campbell *et al.*, 2004; de Mareuil *et al.*, 2005; Giunta *et al.*, 2009).

It has been demonstrated that Tat interacts with LIS1, a microtubule-associated protein that facilitates microtubule polymerization, *in vitro* and *in vivo* (de Mareuil *et al.*, 2005; Epie *et al.*, 2005). Interestingly, the ability of different Tat variants to enhance microtubule polymerization correlates with their capacity to induce apoptosis. It has been shown that Tat interference with the microtubule polymerization results in mitochondrial membrane permeability. Similar to microtubule damaging agents, the pro-apoptotic effect of Tat correlates with the induction of cytochrome *c* release from mitochondria, a critical event triggering apoptosis (Giacca, 2005).

Tat has also been shown to upregulate tumour necrosis factor-related apoptosis-induced ligand (TRAIL), and caspases-3 and -8 in human monocytes (Miura *et al.*, 2003; Zheng *et al.*, 2005). TRAIL is a member of death-inducing ligands of the tumour necrosis factor (TNF) family and activates caspases-3 and -8, resulting in apoptosis (Perez-Cruz *et al.*, 2007; Siegelin *et al.*, 2009; Subramanian *et al.*, 2009). Tat also upregulates Fas, Fas ligand (FasL/CD95L) and TNF- α in human astrocytes (Chauhan *et al.*, 2003). The Fas/FasL system comprises an important mechanism for regulating apoptotic cell death (Inoue *et al.*, 2007; Nakajima *et al.*, 2008). The ability of Tat to recruit so many apoptosis-inducing proteins suggests that Tat induces apoptosis by adopting multiple mechanisms (Dabrowska *et al.*, 2008; Zhao *et al.*, 2007).

The interaction between Tat and the microtubular network may explain the occurrence of neuropathogenesis asso-

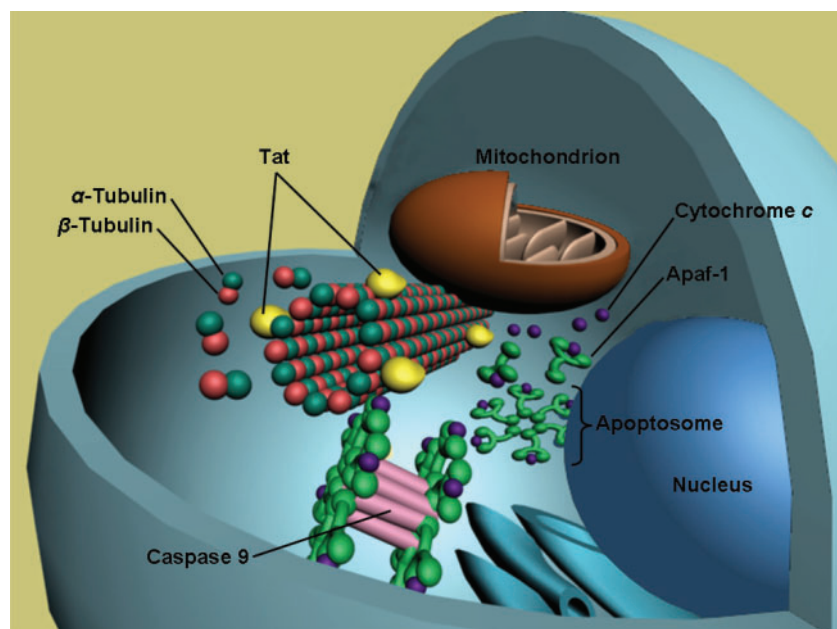


Fig. 3. Apoptosis induction by Tat through altering microtubule dynamics. In addition to the upregulation of apoptotic proteins, Tat triggers the intrinsic pathway of apoptosis by interaction with microtubules and microtubule-associated stabilizing proteins. Microtubules are closely associated with mitochondria for transport and regulation of mitochondrial distribution. Altering the stability of microtubules seriously affects the functions of mitochondria such that they release pro-apoptotic proteins such as cytochrome *c*. Cytochrome *c* molecules interact with apaf-1 molecules, leading to apoptosome formation. Two apoptosomes are linked to one another by caspase-9 molecules. This complex triggers the caspase cascade, resulting in apoptosis.

ciated with the progression of HIV-1 infection, since many human neurodegenerative conditions are accompanied by a reorganization of the neuronal cytoskeleton (Drewes *et al.*, 1998). The induction of the microtubule stabilization by Tat could also be a potentially pro-apoptotic mechanism along with the recruitment of other apoptosis-inducing mechanisms, such as the upregulation of Fas, FasL, TRAIL, Bax and caspase-8 (Chauhan *et al.*, 2003; Giacca, 2005).

Apoptosis inhibition by Tat

Although apoptosis inhibition by Tat appears in conflict with its role in apoptosis induction, it is important to remember that the outcome of Tat activity is dependent on the concentration of Tat, the cell types involved and whether activity is mediated by extracellular or intracellular Tat (Cantaluppi *et al.*, 2001; Mischiati *et al.*, 1999). Tat discriminately acts on different cell types in a concentration-dependent manner such that it has been suggested that the establishment of HIV-1 latent infection could be due to the low levels of Tat production (Deregibus *et al.*, 2002; Gallo, 1999).

Tat has been implicated in the upregulation of B-cell lymphoma 2 (BCL-2), an anti-apoptotic protein, in monocytes isolated from peripheral blood. Extracellular Tat proteins can be taken up by monocytes, and thereby induce biological effects. Since BCL-2 inhibits apoptosis induction by TRAIL, the upregulation of BCL-2 by Tat protects human monocytes from apoptosis (Zhang *et al.*, 2002; Zheng *et al.*, 2007). The anti-apoptotic activities of Tat may account for the establishment of HIV-1 latency in human cells (Deregibus *et al.*, 2002; Zheng *et al.*, 2007). The role of Tat in apoptosis becomes more complicated in the CNS. Despite the adverse effects of Tat protein on the CNS, it has been shown that Tat binds to Bcl-X_L, an anti-apoptotic member of the Bcl-2 family, and forms a complex capable of inhibiting apoptosis in neuronal cells both *in vitro* and *in vivo* (Pugliese *et al.*, 2005).

Cantaluppi *et al.* (2001) have shown that Tat, in a dose-dependent manner, protects Kaposi's sarcoma (KS) cell lines and human umbilical vein endothelial cells from apoptosis induced by vincristine or serum starvation, respectively. Their results indicate that the anti-apoptotic activity of Tat was independent on the modulation of Fas or Bax expression. In contrast, Tat upregulated Bcl-X_L and induced a relevant decrease in caspase-3 activity. Deregibus *et al.* (2002) have shown that Tat protects vincristine-treated KS cells from apoptosis and from the down-regulation of several anti-apoptotic genes such as *akt-1*, *akt-2*, *bcl2* and *bcl-x_L*. Novel studies have shown that TAR miRNA also downregulates cellular genes involved in apoptosis. TAR miRNA has been shown to protect cells from apoptosis by downregulating ERCC1 and IER3. ERCC1 is involved in the detection and base excision repair of damaged nucleotides. IER3 is upregulated after cellular insult and has been shown to be required for the induction of apoptosis after DNA damage (Klase *et al.*,

2009). The anti-apoptotic property of TAR may explain how infected cells can inhibit apoptosis, while extracellular Tat induces apoptosis in bystander cells.

Tat and AIDS-associated cancer

Cancer in general is closely related to genomic instability and DNA repair pathways. DNA repair pathways maintain genomic stability and act as a cellular barrier against cancers. Any agent causing defects in DNA repair pathways could result in cancer (Bernard-Gallon *et al.*, 2008; Bowater *et al.*, 2009). AIDS is accompanied by a significant increase in the incidence of neoplasms. Several reasons have been proposed for this phenomenon, including immunodeficiency, oncogenic DNA viruses and HIV-1 Tat. It has been suggested that the Tat-induced DNA repair deficiencies may be involved in the development of AIDS-associated cancer (Nunnari *et al.*, 2008).

Accumulating evidence indicates that cancer patients with HIV-1/AIDS have more severe tissue reactions and often develop cutaneous toxic effects when subjected to radiotherapy. *In vitro* studies using Tat-expressing cell lines suggest that Tat affects cellular responses to ionizing radiation. The expression of Tat protein in human cell lines may increase the proliferation activity of the cells but decreases their capacity to repair radiation-induced DNA double-stranded breaks (Sun *et al.*, 2006).

An increased oxidative stress level is observed in AIDS patients. A relationship between Tat-induced damage and redox alteration in non-intestinal epithelia has been observed. *In vitro* studies have indicated that exposure to Tat induces oxidative stress in cell cultures which can be partially inhibited by antioxidants (Penza *et al.*, 2008). The role of Tat in the induction of oxidative stress will be discussed in more detail in the next section.

Tat is released by acutely infected HIV T cells and the extracellular Tat promotes the adhesion, migration, invasion and growth of KS and endothelial cells activated with T helper-1 type inflammatory cytokines. In HIV-1-infected individuals, KS is more frequent and severe. Several studies have suggested this severity is due to the HIV-1 Tat which acts synergistically with basic fibroblast growth factor (bFGF) to induce angiogenesis, vascular permeability and oedema. These latter activities are the main processes involved in KS progression (Toschi *et al.*, 2006). The abundance of KS in HIV-infected individuals has also been attributed to the role of Tat in facilitating the replication of KSHV. AIDS-KS is one of the most common AIDS-associated malignancies in HIV-infected homosexual males. KSHV has been described as the aetiological agent of KS. HIV-1 Tat has been implicated to promote KSHV transmission. Tat enhances the entry of KSHV into endothelial and other cells. It has been suggested that Tat could cause KS by facilitating the cells to infection by the KSHV (Aoki & Tosato, 2004; Tian *et al.*, 2008). An increase in the prevalence of anogenital human papillomavirus

(HPV) infection has also been observed in HIV-infected individuals. The virus represents more aggressive behaviour of invasive cervical cancer in these patients. Tat has been shown to transactivate the HPV LCR, stimulating the invasion of the virus which is a potential carcinogen (Nyagol *et al.*, 2006; Turner & Palefsky, 1998).

Role of Tat in oxidative stress, neurotoxicity and HIV-associated dementia (HAD)

Reactive oxygen species (ROS) are naturally generated in cells as by-products of electron transport in mitochondria and redox enzyme reactions in the cytoplasm. Oxidative stress induced by ROS is suspected to be a major cause of chronic neurodegenerative diseases (Toda *et al.*, 2007). Oxidative stress has been demonstrated both in the brain and in cerebrospinal fluid (CSF) of HAD patients (Turchan *et al.*, 2003). The results of some studies suggest that Tat is a potential contributor to HIV-1 dementia (Haughey & Mattson, 2002; Wallace *et al.*, 2005). Tat increases intracellular Ca^{2+} in neurons, followed by mitochondrial calcium uptake which results in the generation of ROS, activation of caspases and eventually apoptosis of neurons. Tat can be transported efficiently across the intact blood-brain barrier. The mRNA levels for Tat have been reported to be elevated in the brain extracts from HIV-1 dementia patients. The Tat sequences from brains of these patients are mutated with glutamate substitutions in the second exon. These mutations may decrease the ability of Tat to enter cells, thus increasing the concentrations of the extracellular Tat proteins and possibly associated neurotoxic effects on the cells. Brain tissues are particularly susceptible to Tat toxicity (Pocernich *et al.*, 2005).

Tat has been reported to upregulate the prostate apoptosis response-4 (Par-4) expression in neurons to induce apoptosis by oxidative stress, which is followed by mitochondrial dysfunction and perturbation of Ca^{2+} -regulated channels and glutamate receptors. Evidently, Tat plays an important role during neuropathogenesis, both as an intracellular and extracellular mediator of neurotoxicity (Wong *et al.*, 2005). Price *et al.* (2005) have demonstrated that Tat causes oxidative stress in immortalized rat brain endothelial cells by a time-dependent decrease in the levels of intracellular glutathione and a time-dependent increase in the levels of the oxidized form of glutathione (GSSG). Glutathione is a critical factor responsible for maintaining the cellular redox status and antioxidant defence in endothelial cells against oxidative stress. As a result of the oxidative stress induced by Tat, glutathione is oxidized to GSSG.

Tat induces its neurotoxicity through interaction with the neuronal cell membrane (Cheng *et al.*, 1998). Tat has been shown to specifically bind to rat brain synaptosomal membranes with moderate affinity (Pocernich *et al.*, 2005). Tat has been shown to enter neurons through binding to the LRP on neurons (Liu *et al.*, 2000). Tat has also been implicated in the induction of neurological dysfunction at sites distant from that of viral replication by neuronal

transport. Extracellular Tat can be transferred from the brain to the spinal cord by CSF, which explains how Tat can cause neurotoxicity and dysfunction at sites distant from viral replication (Pocernich *et al.*, 2005).

Concluding remarks

The modulation of biological pathways by Tat can potentially affect many aspects of infected and non-infected cells. Some of the symptoms associated with AIDS may be due to the direct or indirect activities of Tat. For example, although it is believed that immunosuppression induced by HIV-1 provides the opportunity for opportunistic pathogens, Tat-induced gene transactivation itself facilitates the invasion of several viruses (Aoki & Tosato, 2004; Nyagol *et al.*, 2006; Turner & Palefsky, 1998). It seems logical to hypothesize that in addition to HIV-1-induced immunosuppression, the transactivation of cellular and viral genes by Tat alters conditions in favour of certain pathogens, as well as neoplastic disorders. However, the biological significance of supporting opportunistic pathogens by Tat remains to be elucidated.

Identification of the critical role of Tat in transcription of the HIV-1 genome has provoked a number of studies which focus on Tat as a target for antiretroviral drugs (Daelemans *et al.*, 2000; Melekhovets *et al.*, 2001; Turpin, 2003). The interaction of Tat with TAR is considered a suitable target for the chemotherapy of HIV infection, because an inhibitor of the Tat-TAR interaction may have the potential to maintain the virus in a latent state. A basic peptoid oligomer of nine residues, known as CGP64222, can effectively compete with Tat for binding to TAR and block HIV-1 replication in peripheral blood lymphocytes (Chen *et al.*, 2009; Daelemans *et al.*, 2000). Several studies have developed short TAR RNA decoy molecules which can compete with TAR for binding to Tat (Liang & Wainberg, 2002). The ability of Tat to cross the cellular membrane has also been addressed as a mechanism to deliver new drugs, polypeptides or antibodies to target cells. Several studies have shown that different substances conjugated to Tat or the nuclear import sequences of Tat can cross the cellular membrane (Hu *et al.*, 2006; Schwarze & Dowdy, 2000). These findings may have important therapeutic implications for the future.

Tat could have different effects on different cells, thereby the modulating effects of Tat on gene expression need to be addressed in different cells and tissues. An understanding of the mechanism by which HIV-1 Tat modulates gene expression in HIV-1-infected cells, may reveal the detailed mechanism by which HIV suppresses the immune system. In addition the study of the interactions of Tat with the CNS may lead to novel therapies to control inflammatory processes during the development of HIV-induced neurological diseases. There are still unanswered questions about Tat that need to be addressed. For example, what amounts of Tat are required in different cells to sustain HIV-1 replication and what factors define the amount of Tat

present in cells? Is the concentration of Tat for binding to tubulin and exerting its pro-apoptotic effects compatible with its concentration in the infected cells to diffuse to neighbouring cells? Answers to these and other questions will hopefully be revealed through current research, leading to a better and more comprehensive understanding of this protein.

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