A Novel HIV-1 Therapeutic Target: Tat Transactivator Protein

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Abstract Tat is a HIV-1 transaction transcriptional activator protein and plays a pivotal role in viral replication and in several AIDS-associated pathologies. Its biological properties and functions make it as a good candidate for the development of an anti-AIDS vaccine and/or drug. Strategies designing vaccines and drugs for anti-AIDS include vaccines derived from Tat, extracellular Tat-binding antagonists, inhibitors of Tat-activated intracellular second messengers, anti-Tat antisense, anti-TAR antisense, decoy and antagonists, anti-Tat intracellular intrabody, inhibitors of intracellular Tat cofactors and so on. An effective anti-AIDS therapy will require a multi-targeted approach in which classic antiviral drugs and protease inhibitors are combined with novel extracellular and intracellular Tat antagonists. This approach could prevent the development of drug-resistant HIV strains and decrease the dosage and related toxicity of each single drug and lead to a cure for AIDS-associated pathologies.

Key words Tat, transactivator protein, therapeutic target, anti-AIDS drugs

Many features make HIV peculiar among human viruses and HIV infection difficult to cure. The high levels of viremia found in the initial acute HIV infection quickly decline as the immune response develops. But latent infection persists throughout the long period latency. Under the pressure of immune response, the initial homogeneity of the virus is replaced by rapid appearance of different viral strains. This represents the main obstacle to classical vaccine approaches to AIDS treatment. Indeed, antibodies directed toward HIV structure proteins cannot take into account the great epitope diversity existing among and within infected individuals. Thus, anti-AIDS therapies have been mainly on inhibitors of HIV replication (highly active antiretroviral therapy [HAART]), which have significantly decreased AIDS mortality in the U. S. However, because the virus latently resides in resting memory $CD4^+$ cells and cannot be completely eradicated, HAART must be administered chronically increasing its cost and leading to the development of highly drug resistant HIV strains. Moreover, HAART is frequently discontinued by patients as a drug holiday or because of drug intolerance thus allowing for the reactivation of HIV-1 and progression of AIDS. Therefore, novel anti-AIDS therapies should control AIDS progression and cure associated pathologies rather than eradicate HIV itself. Moreover, the cost anti-AIDS drugs must minimized due to the dramatic increase of AIDS infection in the third world[1].

Tat plays a pivotal role in HIV replication and in several AIDS-associated pathologies and is also implicated in the massive initial viral output that correlates with unfavorable prognosis and to the development of HIV mutants that overwhelm the immune system and /or vaccination[2]. Also, Tat is secreted and is characterized by high amino acid conservation[2]. Interestingly, a stretch of repeated Arg and Lys residues (basic domain) occurs in the Tat protein and is implicated in several aspects of Tat biology. It drivers nuclear and nucleolar delivery of Tat, mediates the interaction of Tat with some cell surface receptors and nucleic acids and is required for some of the biological activities of extracellular Tat[3]. The basic domain is a highly immunoreaction region and is well conserved among Tat proteins isolated from different HIV-1 strains[2]. These characteristics make Tat as a good candidate for the development of an anti-AIDS vaccine and/or drugs[2].

1 Tat structure and function

1.1 Structure of Tat

The Tat gene has been extensively mutagenized[4]. Four domains within the first exon have been identified: acidic, cysteine-rich, core and basic domains. The first three regions are grouped together functionally as an activation domain that has been shown to interact with cellular protein[5] (Figure 1). The Tat activation domain is required for both efficient HIV-1 gene expression and reverse transcription. Mutations in the Tat activation domain severely impair HIV-1 replication. The basic domain, also referred to as the arginine-rich motif (ARM), contains a nuclear localization signal (NLS) and also facilitates the binding of Tat to TAR RNA that is crucial for Tat-mediated transactivation of transcription[6].

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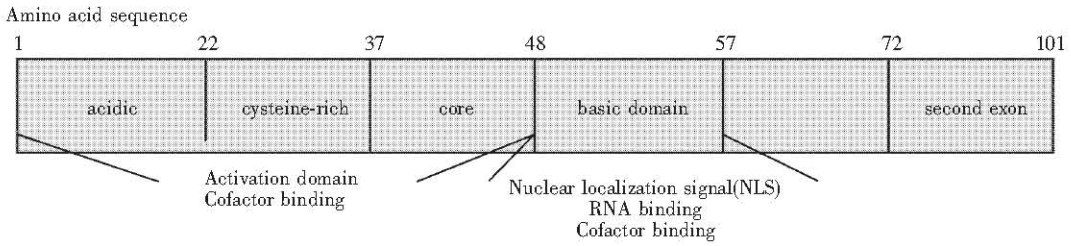

Fig. 1 Structure of the Tat protein

1.2 Function of Tat

The HIV-1 Tat protein is essential for regulation of HIV-1 gene expression. Although the HIV-1 LTR contains binding sites for a variety of transcription factors including NF-κB, SP-1, and TBP, gene expression from the HIV-1 LTR is relatively inefficient because RNAP Ⅱ does not efficiently elongate through its genome[7]. The reason that RNAP Ⅱ elongation is relatively inefficient as it transverses the HIV-1 genome remains to be determined. The Tat protein in conjunction with TAR RNA overcomes the structure constraints of HIV-1 LTR to allow efficient transcriptional elongation though the 9.0 kb HIV-genome. Tat expression leads to 100 to 1 000 fold increase of HIV-1 gene expression and viral replication[8].

2 Extracellular Tat receptors

Extracellular Tat also acts on different types of uninfected cells by interacting with several receptors including integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$; the vascular endothelial growth factor (VEGF) receptors VEGFR1/Fit-1 and VEGFR2/KDR; and the chemokine receptors CCR2 and CCR3 and CD26 (also known as dipeptidly peptidase IV)[9]. Recently, Tat has been shown to also bind the chemokine receptor CXCR-4, the low density lipoprotein receptor-related protein (LPR) and heparan sulfate proteoglycans (HSPGs)[10].

Given the variety of receptors bound by extracellular Tat, it can be expected that a complex network of signal transduction pathways must be activated by the transactivating factor in target cell[9]. Also LPR and HSPG receptors mediated the internalization of Tat inside the cell. Internalized Tat retains the capacity to transactivate viral and /or cellular genes.

3 Intracellular Tat-TAR RNA interaction

TAR RNA has an extensive secondly structure including a stem, a bulge, and a loop[11]. Both for Tat binding and for transcription, there are sequence specific requirements for the immediate stem nucleotide pairs that flank the bulge. A recent series of studies have provided physical insights on how Tat interacts with TAR RNA (Figure 2). The findings indicated that amino acid 41 of Tat lies in close proximity to U42 in the lower TAR stem, that amino acid 47 of Tat is proximal to the G26 nucleotide positioned immediately above the TAR bulge, and that amino acid 57 of Tat is close to U31 in the TAR loop. At the same time, the basic domain of Tat was shown to interact with RNA residues U23, U38, and U40 and to distort /widen the major groove of TAR RNA[12].

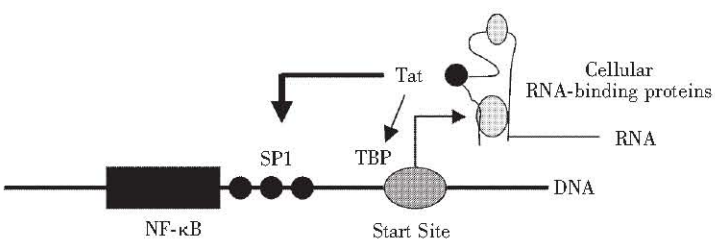

Fig. 2 Interaction of Tat with DNA and RNA targets in the HIV-1 LTR

A schematic representation of the function interaction between Tat, TAR-RNA-binding proteins and promoter elements.

4 Strategies for designing vaccines and drugs

4.1 Tat as vaccine

More than 60 phase Ⅰ/Ⅱ trials of up to 30 candidates AIDS vaccines have been conducted thus far. Accordingly, up to 600 articles have been published about AIDS vaccines. Erroneously suggesting that this kind of cure is at hand. Unfortunately, this may not be true. In fact, the most promising AIDS

vaccine, one directed against the gp120 protein, was recently demonstrated to be ineffective and harmful to human[13]. For many years Tat was not considered a candidate target for an AIDS vaccine because of its intracellular localization. However, the discovery of an extracellular role for Tat would enable use of a strategy for AIDS vaccination that is radically different from previous ones. This new vaccine would be similar to toxoid vaccines for diphtheria or tetanus in that it could be directed against a vial product implicated in the pathogenesis of disease rather than against the viron itself. Cafaro *et al.* vaccinated cynomolgus monkeys with a native Tat protein and obtained complete protection against the highly pathogenic simian-human immunodeficiency virus (SHIV)-89.6P strain in 5 of 7 animals.

Despite its potential therapeutical implications, a Tat vaccine might not be sufficient to cure AIDS. More likely, Tat should be considered as a constituent of a composite vaccine. Vaccination with cDNA encoding for Tat and other HIV protein are under study.

4.2 Extracellular Tat-binding antagonists

Tat has highly binding affinity to heparin and HSPG ($K_d \approx 10$ nmol/L)[10] that suggests polyanionic heparin-like compounds could be used to sequester the extracellular Tat. Polysulfonated suramin, distamycin polysulfate, dextrin-2-sulphate and pentosan polysulfate (PPS)[14] demonstrate extracellular Tat antagonists, which inhibit cell interaction and biological events induced by extracellular Tat. PPS interacts with Tat protein with a 10-fold higher affinity than that of heparin. Accordingly, PPS is highly effective in preventing the interaction of Tat with target cells and in inhibiting the LTR-transactivating and mitogenic activity of tat *in vitro*. Also, PPS inhibits Tat-induced neovascularization *in vivo*[14]. Recently, it was shown that it is possible to produce biotechnological heparin-like molecules by controlled sulfation of the *Escherichia coli* K5 polysacchride. These products have been demonstrated to interact with FGF2 and to inhibit its biological activity *in vitro* and *in vivo*.

4.3 Inhibitors of Tat-activated intracellular second messengers

The interaction of extracellular Tat with cell membrane receptors activates a variety of intracellular signaling pathways and some second messengers appear to be activated by intracellular Tat.

The MAPK $ERK_{1/2}$ is activated by extracellular Tat in many different cell types and may be located at the convergence of the signal transduction pathways activated by the interaction of Tat with KDR and integrins. In microglia, 17β-estradiol suppresses Tat-induced MAPK activation and consequent phagocytosis and superoxide/TNF-α release[15].

Arachidonic acid metabolism can be activated with Tat. Uinacine and chloroquine inhibit the LTR-transactivating activity of Tat[16]. Tat also induces the production of phosphatidic acid. The specific inhibitor CT-2576 prevents the transactivating activity exerted by Tat in epithelial cells and HIV replication in promonocytic cells[17].

In conclusion, specific inhibitors of several second messengers can be used to inhibit different biological activity of Tat. In addition to the possibility of identifying effective Tat antagonist, these studies could help to elucidate the signal transduction pathways responsible for the biological effect of extracellular Tat in target cells.

4.4 Anti-Tat antisense

Gene therapies based on Tat antisense cDNA have been suggested to be capable of blocking the translation of Tat mRNA in HIV-infected cells[18]. Transfection with Tat antisense cDNA efficiently inhibits HIV infection in T lymphocytes and monocytes[19]. This inhibition can also be obtained in the presence of high multiplicity infections and inflammatory cytokines.

Ribozymes are metalloenzymes that retain the properties of an antisense RNA with the additional capacity of catalytic cleavage of specific RNA sequences[20]. Ribozymes directed against Tat mRNA and expressed in target cells by plasmid transfection or retroviral infectin effectively inhibit HIV replication[21]. Ribozymes were also shown to exert their Tat inhibitory effect on primary drug-resistant HIV strains with no mutations or loss of sensitivity to the ribozyme observed in a series of HIV passages in ribozyme-expressing cells[21].

Also relevant to anti-Tat gene therapy is the fact that hematopoietic stem cells, as opposed to circulating cells, can be transfected with anti-HIV genes without altering their differentiation. Theoretically, this could lead to the reconstitution of immune function in AIDS patients. Anti-HIV genes can be engineered under the control of Tat-dependent HIV-LTR promoter to drive their expression only in HIV-infected cells.

4.5 Anti-TAR antisense, decoy and antagonists

Tat stimulates HIV-1 transcription elongation by interacting with a stem-loop RNA element (TAR) formed at the extreme 5′ end of all viral transcripts. Thus, the Tat-TAR complex is considered an important target for anti-HIV strategies. Specific antisense oligonucleotides complementary to the TAR apical stem-loop block Tat binding *in vitro*[22]. The inhibition of Tat-TAR interaction can also be obtained by means of a TAR RNA decoy that blocks the binding of Tat to the authentic TAR region. When expressed in target cells, the TAR decoy significantly reduces HIV infection[23].

Tat-TAR interaction can also be prevented by

means of transdominant Tat protein or Tat peptides. Over-expression of suitable Tat mutants inhibits the transactivating activity of Tat and HIV production in various cell types[24]. Using a combinatorial library, the peptide based compound CGP-642222 that mimics the basic peptide of Tat and binds to TAR, thus preventing Tat transactivating activity and HIV replication in human lymphocytes[25]. Based on peptide models of TAR RNA binding, a set of novel aminoglycoside-arginine conjugates (AACs) have been designed and synthesized. The AACs play an important role, not only as HIV-1 RNA binders but also as inhibitors of viral enter into human cells[26, 27]. Similar results were obtained in our laboratory (Liang *et al.* unpublished observations) with the polyarginine conjugates.

TAR RNA structure-based design methodology in the design of novel RNA or protein-binding drugs has been investigated how the use of medicinal chemistry and structural analysis can provide a rational basis for prediction of ligand-induced conformational change[28].

4.6 Anti-Tat intracellular intrabody

Intracellular antibodies (intrabodies) bind and inactivate intracellular antigens when expressed intracellular in transfected cells. The expression of anti-Tat intrabodies in HIV-infected cells exhibits the promising HIV antagonist effects. Moreover, the structure of anti-Tat intrabodies is to be genetically manipulated to increase their Tat antagonist activity[29].

4.7 Inhibitors of intracellular Tat cofactors

Several intracellular cofactors are required for the nuclear delivery of Tat protein and for its interaction with TAR-RNA. A cyclic peptidomimetic compound that functionally mimics the basic domain of Tat was developed by Friedler *et al.*[30]. This compound blocks the interaction of intracellular Tat with importin-β and its nuclear accumulation. The effect is specific since the peptidomimetic does not influence the nuclear delivery of other nuclear proteins.

P-TEFb is an intracellular complex composed of cyclin-dependent kinase (Cdk) 9 and cyclin T1. Tat-P-TEFb interaction is required for the transactivation of HIV promoter. RNA interference, induced by transfection of small interfering RNA (siRNA) duplexes specific for the essential human immunodeficiency virus type 1 (HIV-1) Tat transcription factor or specific for a cellular coreceptor, CCR5, are shown to protect cell against HIV-1 infection in culture by inducing selective degradation of their target mRNA species[31].

5 Conclusions

Tat could be considered a target for the development of anti-AIDS therapies. Because it was believed to be the main HIV transactivator with an intracellular mechanism of action. AIDS has long been treated as a classic infectious disease with therapies aimed at eradicating its etiological agent. It is now clear that this strategy is not successful and that an effective anti-AIDS therapy will require a multitargeted approach in which classic antiviral drugs and protease inhibitors are combined with novel extracellular Tat antagonists. This approach should prevent the development of drug-resistant HIV strains, decrease the dosage and related toxicity of each single drug and lead to a cure for AIDS-associated pathologies.

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一个新的 HIV-1 治疗靶
——Tat 转录激活蛋白

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摘要 Tat 是 HIV-1 病毒进行转录和复制的一个十分重要的蛋白质，同时，Tat 也与 HIV-1 感染引起的严重病理学程度密切相关. Tat 的生物学性质和功能决定了其是一个理想的开发抗 AIDS 疫苗和药物的靶蛋白. 基于 Tat 自身及其作用的 TAR RNA，可以设计 Tat 疫苗、细胞外结合 Tat 的拮抗剂、抗 Tat 的反义核酸、抗 TAR 的反义核酸、抗 Tat 的细胞内抗体和细胞内 Tat 协同因子的抑制剂等. 传统的抗病毒药物及蛋白酶抑制剂与新的细胞内和细胞外 Tat 拮抗剂联合使用，多靶点地抑制 HIV-1 的复制将是一个有效的抗 AIDS 的治疗方案. 这一治疗方案能够防止 HIV 病毒耐药株的产生，减少单一作用靶点药物的用药剂量和降低相应的毒性，最终治愈 AIDS 相关的病理学变化.

关键词 Tat，转录激活蛋白，治疗靶，抗 AIDS 药物
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