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STAT3 and its Phosphorylation is Involved in HIV-1 Tat-Induced Transactivation of Glial Fibrillary Acidic Protein

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Abstract

Human immunodeficiency virus type 1 (HIV-1) Tat protein is a major pathogenic factor in HIV-associated neurological diseases; it exhibits direct neurotoxicity and indirect astrocyte-mediated neurotoxicity. We have shown that Tat alone is capable of activating glial fibrillary acidic protein (GFAP) expression and inducing astrogliosis involving sequential activation of early growth response protein 1 (Egr-1) and p300. In this study, we determined the roles of signal transducer and activator of transcription 3 (STAT3) in Tat-induced GFAP transactivation. STAT3 expression and phosphorylation led to significant increases in GFAP transcription and protein expression. Tat expression was associated with increased STAT3 expression and phosphorylation in Tat-expressing astrocytes and HIV-infected astrocytes. GFAP, Egr-1 and p300 transcription and protein expression all showed positive response to STAT3 and its phosphorylation. Importantly, knockdown of STAT3 resulted in significant decreases in Tat-induced GFAP and Egr-1 transcription and protein expression. Taken together, these findings show that STAT3 is involved in and acts upstream of Egr1 and p300 in the Tat-induced GFAP transactivation cascade and suggest important roles of STAT3 in controlling astrocyte proliferation and activation in the HIV-infected central nervous system.

Keywords

Astrocytes; HIV-1 Tat; Egr-1; p300; GFAP; STAT3

INTRODUCTION

Introduction of combination antiretroviral therapy has greatly prolonged the life span of HIV-1 infected individuals [1]. Unfortunately, few antiretroviral drugs are able to penetrate the blood-brain barrier and suppress HIV replication, HIV-1 infection-associated neurological diseases, particularly the mild cognitive motor disorder (MCMD) has become more prevalent [2, 3]. Meanwhile, a detectable viral load remains in cerebrospinal fluid and

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CONFLICT OF INTEREST

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subcortical structures of the central nervous system [4]. The pathological hallmarks of the MCMD are astrogliosis, neuroinflammation, and loss of neuronal integrity [5]. However, our understanding of the underlying molecular mechanisms remains very limited.

HIV-1 infection of astrocytes has been documented both *in vivo* and *in vitro* [6, 7], and the infection has primarily been characterized as one that is consistent with a restricted form, expressing early multiply spliced HIV-1 gene products such as Nef [8–11], but no structural proteins [12, 13]. Restrictions in astrocytes take place at entry [14, 15], transcription [16–20] and post-transcription [21–25]. When stimulated with pro-inflammatory cytokines tumor necrosis factor- α or interleukin-1 β , or when co-cultured with CD4+ T cells and monocytic cell lines HIV-infected astrocytes release infectious virions [12, 15, 26]. Astrocytes have been shown to play important roles in HIV neuropathogenesis through: (1) HIV evasion into the central nervous system (CNS) at the interface of blood-brain barriers [27–29]; (2) secretion of cytokines/chemokines to attract cell infiltrates into the CNS and facilitate HIV spread among those cells in the CNS [30–33]; (3) astrocyte activation (astrogliosis) and dysfunction (e.g., glutamate metabolism) and production of neurotoxins and cytokines/chemokines by astrocytes to cause neuronal injury [34–43]. Latent HIV infection in the CNS is associated with astrocyte activation, compromised neuronal integrity, and altered expression of epigenetic factors and cytokine/chemokines in the CNS [44].

Tat is essential for HIV transcription and replication (for a review, see [45]). It has also been shown to be important for HIV neuropathogenesis, as it is a potent neurotoxin and causes direct acute neuronal damage in both *in vitro* and *in vivo* [46–52]. Despite the restricted nature, HIV-infected astrocytes have been shown to express abundant HIV early genes such as Tat, Nef and Rev proteins [8, 11, 16, 53], which could not be attributed to non-productive HIV replication in those cells. In addition, Tat possesses a unique property, i.e., release from one cell and uptake by the other cell [54–56]. Consistent with those findings, Tat is present in the CNS of HIV-infected individuals [57] and expressed in and secreted by HIV latently infected astrocytes [58–60]. In addition, Tat also adversely affects neuron survival at a more physiologically relevant concentrations through subtle effects in cytokine expression, excitatory properties, intracellular signaling and lysosomal function in the neurons [50, 61–68]. Importantly, studies from our group and others have shown that astrocytes potentiate Tat neurotoxicity [50, 58, 69–75]. Of note is that Tat expression in astrocytes alone is capable of including pathological and neurobehavioral changes in the CNS reminiscent of HIV-associated neurological disorders [50, 73, 74, 76–79], confirming that astrocyte-mediated Tat neurotoxicity plays important roles in HIV neuropathogenesis. However, the exact underlying molecular mechanisms have not been identified.

We have recently shown that Tat-induced GFAP activation involves sequential activation of early growth response protein 1 (Egr-1) and p300 [80]. In the current study, we further determined the role of signal transducer and activator of transcription 3 (STAT3) in Tat-induced glial fibrillary acidic protein (GFAP) transactivation cascade. We demonstrated that STAT3 was directly involved and acted upstream of Egr-1 and p300 in Tat-induced GFAP expression and was likely to contribute to astrocyte proliferation and increased GFAP expression in the HIV-infected CNS.

MATERIAL AND METHODS

Cells and Cell Cultures

Human astrocytoma cells U373.MG were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, MO), supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 µg/ml streptomycin in a 37°C, 5% CO₂ incubator. Mouse primary astrocytes were isolated from 18.5-day-old embryonic brain tissue of the Tat-inducible transgenic mice described previously [50, 73, 81]. Human primary fetal astrocytes were isolated, similarly to mouse primary astrocytes, from aborted 16-week fetal tissues (Advanced Biosciences Resources, Alameda, CA). Mouse and human primary astrocytes were cultured in F12-K medium (Cellgro, Manassas, VA) containing 10% FBS, 50 U/ml penicillin, and 50 µg/ml streptomycin in a 37°C, 5% CO₂ incubator. Cells were passaged every 3–4 days.

Plasmids

cDNA3, GL3-basic and CMV-β-gal plasmids were purchased from the Clontech (Mountain View, CA). The constitutively active STAT3 mutant plasmid (STAT3c) was purchased from the Addgene (Cambridge, MA). GFAP promoter-driven luciferase reporter GFAP-Luc3 was a gift from the Dr. Mark Brenner of University of Alabama at Birmingham, Birmingham, AL. p300 and STAT3 plasmids were gifts from Dr. Cheng-Hee Chang of University of Michigan, Ann Arbor, MI and Dr. Xin-Yuan Fu of Indiana University School of Medicine, Indianapolis, IN, respectively. Tat expression plasmid and p300 promoter-driven luciferase reporter p300-Luc was previously described [25, 82]. Egr-1 expression plasmid was constructed using a standard PCR-based cloning method using mRNA from 293T cells as the template, cDNA3 as the backbone, and primers 5'-GGG GTA CCA TGG CCG CGG CCA AGG CCG AGA TGC-3' and 5'-CGG AAT TCT TAG CAA ATT TCA ATT GTC CTG GG-3' (EcoR I and Kpn I sites underlined). STAT3 promoter-driven luciferase reporter was constructed using genomic DNA from U373.MG as the template, GL3-basic as the backbone, and primers: 5'-CTA GCT AGC GGG GAC TAT CTA AC-3' and 5'-CCC AAG CTT CCC GGG TCC CAG GC-3' (Nhe I and Hind III sites underlined).

Transfection and Western Blotting

Cells were plated in a 60 mm plate at a density of 1×10^6 /plate and transfected with 8 µg plasmid using the standard calcium phosphate precipitation methods. The transfection efficiency was determined to be about 30% using a GFP-expressing plasmid. Cells were harvested 72 hr post-transfection for protein analysis and luciferase activity assay. Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed in whole cell lysis buffer [50mM Tris.HCl, pH 7.4, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 2mM PMSF, and 1X protease inhibitor cocktail (Roche, Indianapolis, IN)] on ice for 20 min for whole cell lysate preparation. Protein concentration was determined using a Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA), and the optical density at 650 nm was taken using a Bio-Rad iMark microplate reader (Bio-Rad, Hercules, CA). Cell lysates were electrophoretically separated by 10% SDS-polyacrylamide (SDS-PAGE) and blotted using antibodies against GFAP

(Sigma-Aldrich, St. Louis, MO), STAT3, phosphorylated STAT3 (p-STAT3) (Santa Cruz Biotechnologies, Santa Cruz, CA), or β -actin (Sigma-Aldrich, St. Louis, MO), followed by ECL detection and imaging using a Bio-Rad ChemiDoc imaging system (Bio-Rad, Hercules, CA).

RNA Isolation and Semi-Quantitative RT-PCR

Total RNA was isolated using a TRizol Reagent kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Reverse transcription-PCR (RT-PCR) was performed using a Titan One Tube RT-PCR kit (Boehringer Mannheim, Indianapolis, IN) on a PE Thermocycler 9700 (PE Applied Biosystem, Foster City, CA). The primers for Tat were 5'-GTC GGG ATC CTA ATG GAG CCA GTA GAT CCT-3' and 5'-TGC TTT GAT AGA GAA ACT TGA TGA GTC-3'; the primers for the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5'-CTC AGT GTA GCC CAG GAT GC-3' and 5'-ACC ACC ATG GAG AAG GCT GG-3'. The expected sizes for Tat and GAPDH amplification product were 216 and 500 bp, respectively.

Preparation of Pseudotyped HIV-GFP Virus and Infection of Human Primary Fetal Astrocytes

293T cells were transfected with HIV-GFP and HCMV-G plasmids using the standard calcium phosphate precipitation method. The culture medium was replaced with fresh medium 16 hr post transfection. The cells were cultured for 48 hr. The culture supernatants were collected and removed of cell debris by a brief centrifugation, and used as virus stock. Transfection without pCMV-G plasmid was included as a virus control. Human primary fetal astrocytes were infected with HIV-GFP viruses at multiplicity of infection (MOI = 1) in the presence of 8 μ g/ml polybrene at 37°C for 2 hr. The cells were then thoroughly washed with culture medium to remove unbound viruses, cultured for 48 hr, and harvested for protein lysates.

Luciferase Reporter Gene Assay

Cells were washed twice with ice-cold PBS, lysed in 1X cell lysis buffer (Promega, Madison, WI), and incubated at room temperature for 5 min. The clear supernatants were collected following a brief centrifugation. For β -galactosidase activity assay, cell extracts (10 μ l) was mixed with 3 μ l 100X Mg^{2+} solution (0.1 M $MgCl_2$ and 4.5 M mecaptoethanol, 66 μ l 4 ng/ml O-nitrophenyl-D-galactopyranoside in 0.1 M sodium phosphate, pH 7.5 and 201 μ l 0.1 M sodium phosphate, pH 7.5). The mixture was incubated at 37°C for 30 min, and the optical density at 405 nm was taken using a Bio-Rad iMark microplate reader (Bio-Rad, Hercules, CA). The β -galactosidase activity was then used to normalize the transfection variations among transfections for the subsequent luciferase reporter gene assay, which was performed using a luciferase assay system (Promega, Madison, WI). The luciferase activity was quantitated using an Opticomp Luminometer (MGM Instruments, Hamden, CT).

Data Analysis

All experiment data were analyzed by 2-tailed student's *t* test. A *p* value <0.05 was considered to be statistically significant and marked as *, a *p* value <0.01 was considered to be statistically highly significant and marked as **.

RESULTS

STAT3 and its Phosphorylation Transactivated GFAP Expression

STAT3 has been shown to regulate astrogliogenesis from neuron progenitor cells in the CNS development; it involves external signaling such as IL-6, or internal changes such as epigenetic modifications [83, 84]. Thus, we first determined whether STAT3 expression was sufficient to alter expression of GFAP, the cellular marker of astrocytes. Human primary fetal astrocytes were transfected with a GFAP promoter-driven luciferase reporter and STAT3 expression plasmid or a constitutively active STAT3 mutant, i.e., the phosphorylated STAT3 (STAT3c). Compared to the control, expression of both STAT3 and STAT3c led to significant increases in GFAP-promoter-driven luciferase reporter gene expression (Fig. 1A), suggesting that STAT3 expression and its phosphorylation transactivated the GFAP gene transcription. To determine whether the increased transcription would lead to more GFAP protein expression, human primary fetal astrocytes were transfected with STAT3 or STAT3c, GFAP expression was determined by Western blotting. Compared to the control, higher levels of GFAP proteins were detected in the astrocytes transfected with both STAT3 and STAT3c (Fig. 1B). These results indicate that STAT3 overexpression and its phosphorylation (activation) in astrocytes is sufficient to transactivate GFAP expression at both mRNA and protein levels.

Increased STAT3 Expression and Phosphorylation in Tat and HIV-Infected Astrocytes

HIV-1 infection of the CNS is associated with astrogliosis, marked by increased GFAP expression [34, 85]. We have shown that HIV-1 Tat directly contributes to the increased GFAP expression and astrogliosis [73, 86]. Next, we determined the relationship between Tat expression and STAT3 expression and its phosphorylation. We first took advantage of the mouse embryonic astrocytes isolated from the astrocyte-specific doxycycline (Dox)-inducible Tat transgenic mice. The mouse primary astrocytes were treated with and without Dox for 3 days and harvested for STAT3 expression and phosphorylation by Western blotting. Compared to the control, Tat expression in Dox-treated astrocytes led to not only increased STAT3 expression but also increased STAT3 phosphorylation (Fig. 2A). As expected [73, 86], increased GFAP expression was also detected in Tat-expressing astrocytes. Similarly, results were also obtained in Tat-transfected human astrocytoma U373.MG (Fig. 2B) and HIV-infected human primary fetal astrocytes (Fig. 2C). To determine whether Tat had any effects on STAT3 transcription, human primary fetal astrocytes were transfected with an STAT3 promoter-driven luciferase reporter gene and Tat. STAT3 and STAT3c were also included. Compared to the control, Tat expression led to increased STAT3 promoter-driven luciferase reporter gene activity (Fig. 2D). Meanwhile, STAT3 and STAT3c also showed transactivational activity on the STAT3 promoter [87, 88]. These results suggest that Tat expression leads to increased STAT3 transcription, protein expression and phosphorylation.

STAT3 Involvement in the Tat-Induced GFAP Transactivation Cascade

Our previous studies have shown that HIV-1 Tat expression transactivates GFAP expression through up-regulation of Egr-1 and subsequently p300 [80, 86]. Egr-1 has been shown to act downstream of STAT3 [89]. Therefore, we determined whether STAT3 would act upstream of the Tat-induced GFAP transactivation cascade. To this end, human primary fetal astrocytes were transfected with a GFAP promoter-driven luciferase reporter gene with Tat, STAT3, STAT3c, Egr-1, or p300 expression plasmids. Significantly increased the GFAP promoter activities were detected in all transfection (Fig. 3A), confirming that Tat, STAT3, Egr-1 and p300 are involved in GFAP transactivation. Similarly, Egr-1 and p300 promoter-driven luciferase reporter genes were also tested. Expression of Tat, STAT3 and STAT3c all led to significant increases in both Egr-1 and p300 promoter-driven luciferase reporter gene activities (Fig. 3B, C). As expected [86], Egr-1 expression transactivated p300 promoter activity (Fig. 3C). These results indicate that STAT3 likely functions upstream of the Tat-induced GFAT transactivation cascade.

Dose-Dependent Response of GFAP, Egr-1 and p300 Promoter Activities to STAT3

To further determine the relationship between STAT3 expression and transactivation of the GFAP, Egr-1 and p300 promoter activities, human primary fetal astrocytes were transfected with GFAP, Egr-1 or p300 promoter-driven luciferase reporter gene and increasing amounts of STAT3. GFAP promoter-driven reporter gene activity showed a gradual increase, followed by a gradual decrease when STAT3 was increased (Fig. 4A). Meanwhile, the Egr-1 and p300 promoter-driven reporter gene activities exhibited the similar response kinetics (Fig. 4B, C). The bell-shape dose response is reminiscent of Tat-induced GFAP activation and the double-edged sword function of GFAP activation and astrogliosis [80, 86]. These results further support the notion that STAT3 acts upstream of Egr-1 and p300 in the Tat-induced GFAP transactivation cascade and suggest that there is a threshold of STAT3 response to Tat expression.

Knockdown of STAT3 Attenuated Tat-Induced GFAP Transactivation

To further ascertain the importance of STAT3 in Tat-induced GFAP transactivation, human primary fetal astrocytes were transfected with GFAP or Egr-1 promoter-driven luciferase reporter gene, along with Tat expression plasmid, STAT3-specific siRNA, or Tat plus STAT3 siRNA. Compared to the control, STAT3 siRNA expression alone led to significant decreases GFAP promoter activities (Fig. 5A). Nevertheless, STAT3 siRNA expression only caused slight but not significant decreases in Egr-1 promoter activity (Fig. 5B). On the other hand, Tat expression alone activated GFAP and Egr-1 promoter-driven reporter gene expression. Moreover, compared to the Tat expression alone, Tat plus STAT3 siRNA led to significant decreases in Egr-1 and GFAP promoter activities. Similar results were obtained at the protein levels by Western blotting analysis (Fig. 5C). These results provided further evidence that STAT3 is directly involved in Tat-induced GFAP transactivation activation.

DISCUSSION

In this study, we first demonstrated that STAT3 expression and its phosphorylation led to GFAP transactivation and protein expression in astrocytes (Fig. 1). We also showed that

HIV-1 Tat expression increased STAT3 expression and phosphorylation in astrocytes (Fig. 2), which also occurred in HIV-infected astrocytes. Moreover, we provided evidence to suggest that STAT3 likely acted upstream of the Tat-induced GFAP transactivation cascade (Figs. 3, 4). Lastly, we demonstrated that knockdown of STAT3 led to decreases in not only constitutive but also Tat-induced GFAP and Egr-1 transcription and protein expression (Fig. 5). Taken together, these findings suggest that STAT3 is involved in Tat interaction with astrocytes and subsequent changes of astrocyte function.

STAT3 plays important roles in tumorigenesis and tumor progression [90, 91]. Overexpression of STAT3 inhibits cell apoptosis and promotes cell growth and survival [92]. Blockade of JAK-STAT and MAPK signaling decreases cell viability [93]. Egr-1 is the downstream of MAPK pathway [94, 95] and the downstream of STAT3 signaling [89]. But there is no evidence that Egr-1 directly interacts with STAT3. Activated JAK kinase phosphorylates and activates Src homology-2 domain-containing tyrosine phosphatase, which in turn activates Ras-mediated signaling including ERK, one component of MAPK, while ERK is known to function upstream of Egr-1 [96, 97]. Egr-1 activation is dependent on ERK but independent of STAT3 [98–100]. Meanwhile, other studies have shown that JAK-STAT and MAPK signaling pathways, in which STAT3 and Egr-1 are involved, play a synergistic role in regulating cell growth and apoptosis [93]. Interestingly, the promoter sequence analysis revealed that the GFAP promoter contains one putative STAT3 binding site, raising the other possibility that STAT3 could also directly activate the GFAP transcription and protein expression. STAT3 has been linked to other signaling molecules such as SMAD1 by the co-activator protein p300 and subsequently regulates differentiation of neural progenitors to astrocytes [101]. In this study, we showed that both STAT3 overexpression and phosphorylation transactivated Egr-1 and were likely to contribute to Tat-induced astrocytosis, the increased GFAP expression and astrocyte proliferation in the context of HIV-1 infection of the CNS.

Based on our early studies [50, 73, 80, 81, 86] and the current study, we have further modified the following Tat-induced GFAP transactivation cascade (Fig. 6). When Tat is expressed in HIV-infected astrocytes, there are two possible outcomes. One begins with Tat release outside the infected cells [1], followed by uptake back into the cells [2], or interaction with cell surface molecules [3]. Tat interaction with the cell surface molecules leads to STAT3 phosphorylation and dimerization [4], and subsequent nuclear translocation [5]. In the nucleus, STAT3 transactivates Egr-1 [6], Egr-1 transactivates p300 [7], and p300 transactivates GFAP [8]. The other outcome begins with Tat nuclear translocation [9], followed by STAT3 transactivation [11] and phosphorylation [12], where the first and the second pathways merge and eventually contribute to astrocyte proliferation and increased GFAP expression in the context of HIV infection. Alternatively, expression and phosphorylation of STAT3 could directly transactivate GFAP transcription and protein expression [13].

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Fig. 1. STAT3 expression transactivated GFAP expression in astrocytes

A. Human primary astrocytes were transfected with the GFAP promoter-driven luciferase reporter pGFAP-Luc and STAT3, or STAT3c, cultured for 72 hr and harvested for the luciferase reporter gene assay. cDNA3 (C3) was included as the DNA control; pCMV-βgal was used to normalize for the transfection efficiency variations among all transfections. The data were the mean $\pm$ SD of triplicate samples and representative of three independent experiments. Statistical analysis was performed using C3 as the reference. **B.** Human primary astrocytes were transfected cDNA3, STAT3, or STAT3c, cultured for 72 hr and harvested for GFAP, STAT3 and pSTAT3 expression by Western blotting. β-actin was included as a loading control. C3 was included as the DNA control. The data were representative of three independent experiments.

Fig. 2. Tat expression and HIV infection augmented STAT3 expression and phosphorylation in astrocytes

A. Mouse embryonic primary astrocytes from iTat mice were treated with and without Dox for 96 hr and harvested for STAT3, pSTAT3, and GFAP expression by Western blotting, or Tat expression by semi-quantitative RT-PCR. β -actin was included as a loading control for Western blotting, GAPDH was included as loading control for RT-PCR. The data were representative of three independent experiments. **B.** U373.MG were transfected with Tat, cultured for 72 hr, and harvested for Western blotting or RT-PCR. The data were representative of three independent experiments. **C.** Human primary fetal astrocytes were infected with VSV-G pseudotyped HIV-GFP virus. Cells were harvested 48 hr post-infection for Western blotting. HIV-GFP viruses without envelope were included as a control. Arrow: Western blotting with an anti-p24 antibody. The data were representative of three independent experiments. **D.** Human primary fetal astrocytes were transfected with the STAT3 promoter-driven luciferase reporter gene pSTAT3-Luc with Tat, STAT3, or STAT3c. Cells were harvested 72 hr post-transfection for the luciferase reporter gene assay. pCMV- β -gal used to normalize the transfection efficiency variations among all transfections. C3 was included as the DNA control (**B & D**). The data were the mean $\pm$ SD of triplicate samples and representative of three independent experiments. Statistical analysis was performed using C3 as the reference.

Fig. 3. Interaction of STAT3 with the Tat-Egr-1-p300-GFAP transcriptional cascade

Human primary fetal astrocytes were transfected with GFAP (A), Egr-1 (B), or p300 (C) promoter-driven luciferase reporter gene and STAT3, STAT3c, Tat, Egr-1 or p300 expressing plasmid as indicated. Cells were harvested 72 hr post-transfection for the luciferase reporter gene assay. C3 was included as the DNA control. pCMV- β -gal used to normalize the transfection efficiency variations among all transfections. Statistical analysis was performed using C3 as the reference.

Fig. 4. Dose response of GFAP, Egr-1, and p300 transcription to STAT3

Human primary fetal astrocytes were transfected with GFAP (A), Egr-1 (B), or p300 (C) promoter-driven luciferase reporter gene and increasing amount of STAT3 expressing plasmid. Cells were harvested 72 hr post-transfection for the luciferase reporter gene assay. C3 was included as the DNA control. pCMV-β-gal used to normalize the transfection efficiency variations among all transfections. Statistical analysis was performed using C3 as the reference.

Fig. 5. Attenuation of Tat-induced GFAP and Egr-1 activation by STAT3 knockdown

Human primary fetal astrocytes were transfected with GFAP (A) or Egr-1 (B) promoter-driven luciferase reporter gene and Tat, STAT3 siRNA, or Tat plus STAT3 siRNA. Cells were harvested 72 hr post-transfection for the luciferase reporter gene assay (A–B) or Western blotting (C). C3 was included as the DNA control (–); control siRNA was included as a control for STAT3 siRNA (–). pCMV-β-gal used to normalize the transfection efficiency variations among all transfections. β-actin was included as a loading control for Western blotting.

Fig. 6.
Proposed Tat-induced GFAP transactivation cascade with inclusion of STAT3.