HHS Public Access

Author manuscript

J Neurovirol. Author manuscript; available in PMC 2019 April 02.

Published in final edited form as:

J Neurovirol. 2018 April ; 24(2): 168–179. doi:10.1007/s13365-017-0598-9.

Doxycycline-inducible and astrocyte-specific HIV-1 Tat transgenic mice (iTat) as an HIV/neuroAIDS model

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Abstract

HIV-1 Tat is known to be neurotoxic and important for HIV/neuroAIDS pathogenesis. However, the overwhelming majority of the studies involved use of recombinant Tat protein. To understand the contributions of Tat protein to HIV/ neuroAIDS and the underlying molecular mechanisms of HIV-1 Tat neurotoxicity in the context of a whole organism and independently of HIV-1 infection, a doxycycline-inducible astrocyte-specific HIV-1 Tat transgenic mouse (iTat) was created. Tat expression in the brains of iTat mice was determined to be in the range of 1–5 ng/ml and led to astrocytosis, loss of neuronal dendrites, and neuroinflammation. iTat mice have allowed us to define the direct effects of Tat on astrocytes and the molecular mechanisms of Tat-induced GFAP expression/astrocytosis, astrocyte-mediated Tat neurotoxicity, Tat-impaired neurogenesis, Tat-induced loss of neuronal integrity, and exosome-associated Tat release and uptake. In this review, we will provide an overview about the creation and characterization of this model and its utilities for our understanding of Tat neurotoxicity and the underlying molecular mechanisms.

Keywords

HIV-1; Tat; Transgenic; Mouse model; Brain

Introduction

Tat protein is neurotoxic in vitro. A variety of mechanisms have been proposed and include neuron depolarization, increased intracellular calcium, pro-inflammatory cytokine production, immune cell infiltration, activation of excitatory amino acid receptors, and cell

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

death (Kruman et al. 1998; New et al. 1998; Sabatier et al. 1991; Shi et al. 1998). Tat protein, when present in vivo, causes histological changes in the brain that are consistent with those observed in patients with HIV dementia (Jones et al. 1998; Rappaport et al. 1999). However, it is not clear whether there are high enough of Tat protein within the CNS of HIV-infected individuals (1 to 10 μM) to exhibit such an acute neurotoxicity. On the other hand, recombinant Tat protein was shown to alter gene expression and cell growth, adhesion, and morphology at lower concentrations (nM), at which no acute toxic effects were detected on target cells, including neurons (Ensoli et al. 1990; He et al. 1995; Helland et al. 1991; Marcuzzi et al. 1992; Milani et al. 1993).

Several HIV-1 Tat transgenic mouse models have been developed to study the role of HIV-1 Tat protein in HIV/ AIDS pathogenesis (Choi et al. 2000; Garza et al. 1996; Kundu et al. 1999; Nerenberg et al. 1987; Vellutini et al. 1995; Vogel et al. 1988). All of these models were designed to constitutively express Tat in most or all tissues from the beginning of development. Such a design made it difficult, if not entirely impossible, to discern Tat effects on the brain from those on other tissues or organs. Thus, to gain a better understanding of the role of Tat in HIV/neuroAIDS and the mechanisms of Tat neurotoxicity, if any, specifically resulting from Tat protein expression in the brain, the He lab combined the doxycycline (Dox)-regulated (rtTA) gene expression system (Gossen and Bujard 1992) with a brain-specific promoter, i.e., the glial fibrillary acid protein (GFAP) promoter (Mucke et al. 1995; Toggas et al. 1994). A similar strategy has been successfully used to study functions of a number of genes within the CNS at a specific time and in a specific concentration (Chen et al. 1998; Furth et al. 1994; Mansuy et al. 1998; Passman and Fishman 1994). The main reasons to select the GFAP promoter to target Tat to astrocytes in the CNS at that time were: (1) Tat expression in astrocytes would release Tat and allow Tat to interact with neurons in a way that is reminiscent of Tat in the HIV-infected brain; (2) Tat showed no apparent cytotoxicity to astrocytes (Zhou and He 2004); (3) Accumulating evidence suggested that astrocytes play important roles in HIV/neuroAIDS (Benos et al. 1994; Genis et al. 1992; Levi et al. 1993; Saito et al. 1994; Tornatore et al. 1991); (4) This strategy had been proven to be effective in delivering other proteins to the CNS, including HIV-1 gpl20 and AD-related factors such as ApoE4 (Campbell et al. 1993; Johnson et al. 2010; Mucke et al. 1995; Smith et al. 1998; Sun et al. 1998; Toggas et al. 1994); and (5) This strategy had successfully been used to implicate cytokines IL-3, IL-6, IL-12, TNF-α, and IFN-α in the pathogenesis of other CNS diseases (Brenner et al. 1994; Campbell 1998; Campbell et al. 1998; Carr et al. 1998; Raber et al. 1997).

Generation of iTat mouse model

The first step in the generation of the iTat mouse model was to prepare two transgene constructs that contained the essential components (Kim et al. 2003). One was the regulator, pTeton-GFAP, in which the CMV promoter was replaced by a 2.1-kb GFAP promoter (a gift from Dr. L. Mucke) (Fig. 1a). The other was the expressor, pTRE-Tat86, in which HIV-I HXB2 Tat cDNA (86 amino acids in length) was cloned under the control of the tetO promoter. The pTRE-CAT containing the CAT gene was created as an expressor control and used to determine the specificity of the GFAP promoter. pTeton-GFAP and pTRE-CAT DNA was transiently transfected into astroglial U87.MG cells, and non-astroglial HeLa cells to

confirm the specificity of the GFAP promoter activity in astrocytes (Kim et al. 2003). To begin to create the iTat mice, the strategy was to generate two strains of transgenic mice, one for the GFAP-rtTA transgene (G-tg) and one for the TRE-Tat86 transgene (T-tg), and then crossbreed those two strains of mice to obtain the bigenic iTat mice. Both GFAP-rtTA and TRE-Tat86 transgenes were released by restriction digestion (Xho I and Pvu II) from their respective plasmid DNA pTeton-GFAP and pTRE-Tat86, purified, and microinjected into fertilized murine oocytes (C57BL6 xSJL). Five GFAP rtTA transgenic founders and seven TRE-Tat86 transgenic founders were obtained. Backcrossing with wild-type C57BL6 mice was performed to stabilize the GFAP-rtTA transgenic and TRE-Tat86 transgenic lines. Then, GFAP-rtTA mice were crossbred with TRE-Tat86 transgenic mice to obtain iTat mice carrying both GFAP-rtTA and TRE-Tat86 transgenes. The presence of transgenes was confirmed by PCR using genomic DNA from the mouse-tail with transgene-specific primers (Fig. 1b). Homozygous genotypes were confirmed by crossbreeding transgenic mice with wild-type C57/BL6 mice. After successfully obtaining four iTat mouse lines with expression levels from low to high (Kim et al. 2003), an iTat line with Tat expression levels close to that of HIV-infected brains was maintained (Fan et al. 2016; Westendorp et al. 1995; Xiao et al. 2000) and used in subsequent studies and distributed to many other laboratories.

Tat expression in the brain of iTat mice

To determine whether Dox would induce Tat expression in the brain of iTat mice, 21-day-old mice were provided different dosages of Dox-containing drinking water for different periods of time (Kim et al. 2003). Tat expression was initially confirmed using reverse transcriptase (RT)-PCR, and more recently real-time RT-PCR of the total RNA isolated from the brain with Tat gene-specific primers. There was a minimal (leaky) level of Tat mRNA in the brains of iTat mice without Dox treatment (Kim et al. 2003). To ensure that the designed constructs specifically targeted Tat expression within the brain, total RNA was isolated from various organs and tissues of the iTat mice, including the eye, heart, kidney, liver, lung, spleen, and thymus, and Tat expression was determined by RT-PCR. The results indicated that systematic exposure to Dox induced Tat expression only in the brain (Kim et al. 2003). Based on the level of Tat expression, 6 mg/ml Dox in drinking water for 7 days was chosen for the induction. Due to the inconsistency of Dox intake from the drinking water, Dox delivery route was subsequently changed from drinking water to i.p. injection at a dosage of 80 mg/kg/day for 7 days. To determine the expression level of Tat protein in the brain, the HIV LTR promoter-driven luciferase reporter cell line TZM-bl was utilized. TZM-bl cells were treated with serially diluted recombinant Tat protein and the luciferase reporter gene activity assay was performed to establish a linear regression between the level of Tat and the luciferase activity. Then, cells were treated with purified brain homogenates of Dox-treated iTat mice and the luciferase activity was determined. Based on the luciferase activity, Tat protein expression levels in the brains of Dox-treated iTat mice were calculated to be in the range of 1–5 ng/ml (Fan et al. 2016) (Fig. 1c), which is indeed close to the reported Tat levels in the brains of HIV-infected individuals (Fan et al. 2016; Westendorp et al. 1995; Xiao et al. 2000).

Neuropathologies of iTat mice

Among the features that are often observed in HIV-induced neuropathologies prior to and in the era of combination anti-retroviral therapy (cART) are reactive astrogliosis, loss of neuronal integrity (neuronal dendrites), and chronic neuroinflammation. Astrogliosis is a common manifestation of brain pathology in a variety of neurological diseases, although the precise cause(s) remain unknown. Therefore, we determined whether Tat expression in astrocytes would induce astrogliosis. Immunofluorescence labeling of the brain sections using an anti-GFAP antibody indicated that there was widespread reactive astrogliosis in the brains of the iTat mice treated with Dox (Kim et al. 2003; Zou et al. 2007) (Fig. 2), which was reflected by an increase in the number of GFAP-expressing astrocytes. In addition, astrocytes of iTat mice also had a typical reactive morphology, showing increases in both cell body size and cell processes. Moreover, microglia exhibited intense staining and morphological changes in the brains of iTat mice, both of which are typical characteristics of activated microglia (Zou et al. 2007). This finding suggests that microgllia activation may also contribute to Tat neurotoxicity in iTat mice.

Next, it was determined whether Tat expression in astrocytes was sufficient to cause neuronal damage. The integrity of neuronal dendrites in brain sections of the iTat mice treated with Dox was compared to age-matched wild-type control mice using an anti-MAP2 antibody. The results showed a significant loss of neuronal dendrites in the brains of the iTat mice treated with Dox, when compared with the age-matched wild-type controls (Fan and He 2016b; Kim et al. 2003; Rahimian and He 2016b; Zou et al. 2007) (Fig. 2).

Furthermore, the relationship between Tat expression and inflammatory response in the brain was assessed. Compared to the wild-type control mice treated with Dox, iTat mice showed increased infiltration of activated macrophages/monocytes and T lymphocytes into the brain (Kim et al. 2003; Zou et al. 2007). In agreement with those findings, there were increased levels of cytokines and chemokines in the brains of iTat mice including monocyte chemoattractant protein-1 and protein-2, macrophage inflammatory protein-1α and protein-1β, RANTES, inducing protein-10, and lymphotactin/single C motif-1α/activation-induced, and T cell-derived and chemokine-related cytokine (Kim et al. 2004).

Use of iTat mice in studies of Tat neurotoxicity and its molecular mechanisms

Tat expression inhibits astrocyte proliferation

Tat protein exerts various effects on host cells that include changes in the cell cycle (Kundu et al. 1998), T lymphocyte survival (Gibellini et al. 1995; Zauli et al. 1993), and growth of T lymphocytes and enterocytes (Canani et al. 2003; Viscidi et al. 1989). To determine effects of intracellular Tat expression on astrocytes, primary astrocytes were isolated from 16-day-old embryos of iTat mice and treated with Dox to induce Tat expression and then analyzed for proliferation using the [^{3}H] thymidine incorporation assay. Compared to primary astrocytes from wild-type mice, iTat primary astrocytes exhibited significantly slower proliferation and the culture supernatants from iTat astrocytes were neurotoxic (Zhou and He

2004). The anti-proliferative effects of intracellular Tat on astrocytes did not appear to be due to premature cell senescence; instead, it could be attributed to Tat interactions with various cell cycle-related proteins including cyclin A, cyclin B, cyclin D3, cdk2, cdk, and cdk1/cdc2 (Fig. 3).

Tat transactivates GFAP expression

Astrogliosis has been noted in the brains of iTat mice. Meanwhile, intracellular Tat expression results in anti-proliferative effects on astrocytes. These findings raised the possibility that Tat could directly transactivate GFAP expression. To address this possibility, iTat mice or primary astrocytes isolated from iTat mice were used to define a cascade of transcriptional factors that formed the network through which Tat transactivates GFAP expression (Fan et al. 2015, 2011; Kim et al. 2003, 2004; Zhou et al. 2004; Zou et al. 2010) (Fig. 4). The first route is that Tat released from HIV-infected macrophages/microglia and astrocytes interacts with cell surface molecules, induces STAT3 phosphorylation and dimerization, and its subsequent nuclear translocation. The second route is that Tat directly translocates into the nucleus and transactivates STAT3 expression. In both scenarios, phosphorylated STAT3 transactivates Egr-1, transactivates p300 and GFAP, and contributes to increased GFAP expression in the brains of iTat mice and in the brains of HIV-infected individuals.

GFAP activation and Tat neurotoxicity

Abnormal GFAP expression alone has been shown to be sufficient to cause neuropathologies (Gomi et al. 1995; Liedtke et al. 1996; Messing et al. 1998; Pekny et al. 1998). Astrogliosis or increased GFAP expression in astrocytes is one of the very few consistent hallmarks of HIV-1 infection of the CNS (Bell et al. 2006a; Price 1996). Astrocytes have a variety of functions, many of which are related to the CNS homeostasis [see reviews (Barres 1991; Eddleston and Mucke 1993)]. More recent evidence points to a highly dynamic and reciprocal relationship between astrocytes and neurons with a notion that astrocyte dysfunction contributes to neurological diseases. For example, viral infection-induced astrocyte dysfunctions contribute to several CNS diseases (Aksamit et al. 1986; Aubert et al. 1987; Carbone et al. 1991; Itoyama et al. 1991; Rinaman et al. 1993; Stowring et al. 1985). However, the direct link between GFAP activation, astrocytosis, and Tat neurotoxicity was not clear. Using iTat mice or primary astrocytes from iTat mice, studies showed that Tat expression in astrocytes led to marked impairment of glutamate uptake by astrocytes and importantly, that cell culture supernatants derived from Tat-expressing astrocytes induced neurotoxicity (Fitting et al. 2013; Kim et al. 2003; Zhou and He 2004; Zhou et al. 2004). Furthermore, it was shown that disruption and inhibition of GFAP expression abrogated astrocyte-mediated Tat neurotoxicity (Fan and He 2016a, b; Zou et al. 2007). These data show that Tat expression in astrocytes leads to astrocyte dysfunction and subsequent neurotoxicity, and support the notion that astrocyte dysfunction contributes, at least in part, to Tat neurotoxicity and subsequently to HIV/neuroAIDS.

Tat expression causes ER stress in astrocytes and enhances lysosomal exocytosis from astrocytes

Tat expression in astrocytes alone was sufficient to induce pathological changes such as astrocytosis, loss of neuronal dendrites, and neurobehavioral deficits including impaired motor and cognitive functions in the CNS reminiscent of HIV-associated minor cognitive motor disorder (MCMD) (Carey et al. 2012; Fan et al. 2011; Fitting et al. 2013; Kim et al. 2003; Paris et al. 2014c; Zhou et al. 2004) (see the review by McLaughlin, et al. in the same issue). However, the exact underlying molecular mechanisms were not clear. Using combined molecular, cellular, biochemical, and genetic approaches, Fan and He demonstrated that Tat expression led to formation of GFAP aggregates and activation of the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress in astrocytes (Fan and He 2016a). In addition, they showed that UPR/ER stress activation promoted calcium-dependent lysosomal exocytosis from astrocytes and as a result, led to astrocyte-mediated Tat neurotoxicity (Fan and He 2016b). Lastly, these studies demonstrated that the chemical chaperone 4-phenylbutyrate significantly abrogated astrocyte-mediated Tat neurotoxicity through inhibition of Tat-induced UPR/ER stress (Fan and He 2016b). Taken together, these findings support a working model (Fig. 5): Tat from HIV-infected macrophages/microglia is taken up by astrocytes and/or Tat is expressed in HIV-infected astrocytes thereby activating GFAP expression and leading to GFAP aggregation. GFAP aggregation in turn activates UPR/ER stress, calcium release from ER storage into the cytoplasm, and subsequent lysosomal exocytosis from astrocytes and release of neurotoxic lysosomes and their neurotoxic components, such as cathepsins (Fan and He 2016b). These findings provide new and important insights not only about the roles of this critical and pervasive protein Tat in HIV/neuroAIDS, particularly in the era of cART, but also about the general roles of astrocytosis and GFAP up-regulation in contributing to neurodegenerative diseases.

Tat expression impaired neurogenesis

HIV infection of the CNS often leads to cognitive, motor, and neurobehavioral dysfunction (Dube et al. 2005; Epstein et al. 1986; Valcour et al. 2004). Adult neurogenesis in the hippocampus is critical for the maintenance of intact cognitive functions, such as learning and memory, and is regulated by physiological and pathological stimuli (Fuchs and Gould 2000; Leuner et al. 2006; Ming and Song 2005). Impaired neurogenesis has been noted in the hippocampus of HIV-infected individuals, SIV-infected macaques, and severe combined immunodeficiency mice injected with HIV-infected human macrophages/microglia (Curtis et al. 2014; Krathwohl and Kaiser 2004a, b; Poluektova et al. 2005). HIV could have effects on neuron progenitor cells (NPC) through direct infection (Lawrence et al. 2004; Schwartz and Major 2006; Tran et al. 2005; Whitney et al. 2009), proliferation (Krathwohl and Kaiser 2004b; Okamoto et al. 2007), and migration (Belmadani et al. 2005, 2006). Tat protein has been shown to affect mature neurons in a variety of ways (Chen et al. 1997; Cheng et al. 1998; Conant et al. 1998; Hofman et al. 1999; Jones et al. 1998; Zidovetzki et al. 1998) and to inhibit NPC proliferation and differentiation in vitro (Mishra et al. 2010; Yao et al. 2012). Given the recent findings that Tat is persistently expressed in the brain of HIV-infected individuals treated with cART (Johnson et al. 2013), it was imperative to determine the effects of Tat expression on neurogenesis and HIV-associated minor cognitive motor disorder (MCMD). Taking advantage of the iTat mice, researchers showed that Tat inhibited

NPC proliferation and migration and altered NPC differentiation to favor astrogliogenesis over neurogenesis through Tat binding to Notch signaling factors (Fan et al. 2016) (Fig. 6). These findings point to the potential of developing Notch signaling inhibitors as HIV/neuroAIDS therapeutics.

Tat-mediated miR-132 expression and its effects on neuronal dendrites

Successful suppression of active HIV replication by cART has resulted in dramatic decreases of the incidence of HIV-associated dementia from 15 to 2% (Hult et al. 2008) (Ghafouri et al. 2006). However, up to 50% of HIV-infected individuals exhibit a less severe form of HIV-associated neurocognitive disorders (HAND), MCMD (Bell et al. 2006b). Synaptodendritic injury is a positive correlate of MCMD, neuroinflammation, and cognitive impairments of HIV-infected individuals (Adle-Biassette et al. 1999; Masliah et al. 1992, 1997; Moore et al. 2006; Rao et al. 2012, 2011). However, the exact underlying molecular mechanisms of HIV-associated synaptodendritic injury and synaptic loss were largely unknown. Using a combined molecular, cellular, and genetic approach, including iTat mice, Rahimian and He showed that Tat-induced miR-132 expression and that exosomes associated with miR-132 from astrocytes caused neurite shortening, likely through down-regulation of miR-132 target genes such as brain-derived neurotrophic factor (BDNF) and methyl CpG-binding protein 2 (MeCP2) (Rahimian and He 2016b) (Fig. 7). These findings showed that Tat-induced miR-132 expression contributes to both direct and astrocyte-mediated Tat neurotoxicity, and supports the important roles of miR-132 in regulation of neurite outgrowth.

Exosome-associated Tat release and uptake

Biologically active and intact Tat protein is secreted from HIV-infected cells (Westendorp et al. 1995; Xiao et al. 2000), Tat-expressing cells (Albini et al. 1998; Chang et al. 1997; Ensoli et al. 1990; Milani et al. 1993; Morgavi et al. 1997; Zauli et al. 1993, 1995), and is detected in HIV-infected brains (Hudson et al. 2000). Tat does not contain an export signal sequence (Chang et al. 1997; Morgavi et al. 1997). Thus, its secretion occurs through unconventional secretory pathways. Tat or Tat-derived peptides are capable of entering cultured cells and transactivating the HIV LTR promoter (Bonifaci et al. 1995; Frankel and Pabo 1988; Green et al. 1989; Liu et al. 2000; Mann and Frankel 1991; Viscidi et al. 1989). The basic domain of Tat protein has also been utilized to successfully deliver protein cargo into cells (Fawell et al. 1994; Schwarze et al. 1999; Vives et al. 1997). We have demonstrated that Tat specifically binds to low-density lipoprotein receptors on neurons, which leads to Tat neuronal uptake in its biologically active form (Liu et al. 2000). Using primary astrocytes from iTat mice, a HIV-1 LTR-driven luciferase reporter-based cell system and a well-defined OptiPrep-based exosome purification protocol, researchers showed that Tat was secreted in exosomes, which were potently neurotoxic (Rahimian and He 2016a). These findings indicated that a significant fraction of Tat is secreted in the form of exosomes and may contribute to the stability of extracellular Tat and broaden the spectrum of its target cells.

Use of iTat mice in other studies

While many studies in the HIV/neuroAIDS field focus on astrocytosis, neuronal integrity, and neuroinflammation as the outcome measures, there are many other processes that occur

within the brains of HAND patients. The iTat mouse has been utilized to investigate some of these processes. Among the many reports utilizing the iTat model just within the last decade are studies addressing Tau processing (Kadri et al. 2015), host cell cycle regulation (Fields et al. 2015b), cell survival (Fan and He 2016b), differentiation (Perry et al. 2010; Wheeler et al. 2008; Yao et al. 2012) and glial activation (Kiebala et al. 2010), substance abuse (Fitting et al. 2012, 2010; Hauser et al. 2009; Mediouni et al. 2015; Paris et al. 2014a; Zou et al. 2011), sex differences (Hahn et al. 2015a, b), behavior (Hahn et al. 2016; Paris et al. 2014b, c, 2015), memory and learning (Carey et al. 2012; Fitting et al. 2013) and brain changes observed via imaging (Carey et al. 2015, 2013). Table 1, while not comprehensive by any means, provides examples of the diversity of studies that have utilized the iTat mouse model. Continuing studies using the iTat model will open new opportunities for addressing important processes such as cART effects, aging, epigenetics, and therapy development in the context of the Tat protein.

Reversible changes of Tat-induced neuropathologies in iTat mice

cART effectively suppresses HIV replication in the periphery. With a better ability to cross the blood-brain barrier, cART can also suppress HIV replication in the central nervous system and lead to decreases in HAND. However, neuroinflammation rather than viral load appears to be more important to HAND, and there have been no HAND-specific therapeutics. As discussed above, iTat mice have consistently recapitulated astrocytosis, compromised neuronal integrity, and neuroinflammation, three consistent hallmarks of HAND in the era of cART. EGb 761, a standardized formulation of *Ginkgo biloba* extract, has the ability to protect iTat mice from Tat-induced astrocytosis and neuroinflammation through down-regulation of GFAP expression (Zou et al. 2007). Chemical chaperone 4-phenylbutyrate inhibits Tat- or GFAP-induced unfold protein response/endoplasmic reticulum stress and alleviates astrocyte-mediated Tat neurotoxicity in vitro and in the brains of iTat mice (Fan and He 2016a). Treatment of conditioned medium of Tat-expressing astrocytes with anti-cathepsin B antibody led to marked decreases in the neurotoxicity of Tat-expressing astrocytes (Fan and He 2016b). Notch signaling inhibitor greatly improved Tat-impaired NPC differentiation and neurogenesis in iTat mice (Fan et al. 2016). miR-132 inhibitor inhibits astrocyte-derived Tat neurotoxicity, specifically neuron dendritic damage and loss of and synapse formation (Rahimian and He 2016b). Taken together, these findings demonstrate that Tat-induced neuropathologies (likely neurocognitive impairments as well) are reversible and suggest the utility of this model for development of HIV/neuroAIDS therapeutics, particularly those targeted at the early stages of HIV infection of the CNS.

Limitations of the iTat mouse model

Even though transgenic mouse models were among the first developed to study HIV infection, the use of these models has posed numerous challenges since rodents are not natural hosts of HIV and do not support viral replication (Jaeger and Nath 2012). As more knowledge was gained regarding the specific effects of certain viral proteins including gp120 and Tat, and in answer to these biological barriers, viral protein-specific transgenic animals were created.

Recombinant Tat protein when present in the brain causes histological changes that are consistent with those seen in patients with HIV dementia (Jones et al. 1998; Rappaport et al. 1999). However, it is still unclear whether Tat is present in the CNS or cerebrospinal fluid in HIV-infected individuals at sufficient concentrations to directly cause acute neurotoxicity that is observed in the transgenic models. However, the GFAP-driven expression of Tat protein by astrocytes in the brains of iTat mice was close to actual levels in the brains of HAND patients (Fan et al. 2016; Westendorp et al. 1995; Xiao et al. 2000). In vitro cell culture and in vivo studies show that HIV infects astrocytes, but the contribution that this population of cells makes to HAND is unclear (Jaeger and Nath 2012; Kramer-Hammerle et al. 2005). A recent study shows that in individuals with HAND, up to 19% of perivascular astrocytes are infected by HIV (Churchill et al. 2009), which may represent a considerable percentage of infected cells in the CNS. On the other hand, astrocytes are not believed to generate significant amounts of virus, but studies have shown that even in the absence of productive viral replication, Tat is still produced. Moreover, studies have shown that even during successful cART, Tat is generated (Jaeger and Nath 2012).

Another main limiting factor in using iTat mice to study HAND is the fact that Tat is only one factor involved in the neuropathogenic processes in CNS infection with HIV. Many viral proteins including Tat, gp120, gp140, Nef, Vpr, and Rev are also toxic to neurons (Nath 2002), and the iTat mouse model considers only one of these. In this context, different viral proteins target different host cell pathways can, therefore, dysregulate a variety of downstream signaling cascades that may lead to aberrant cell signaling.

One other question remains: How does the iTat model relate to the neuropathology of HAND patients without HIVE treated with cART? With cART, no specific neuropathology has been reported to be linked to HAND (Gelman 2015; Gelman et al. 2013). HAND persists in up to 50% of virally suppressed HIV patients (Heaton et al. 2010) and it could involve other neuronal dysfunctions (Ellis et al. 2007; Gelman 2015). Some studies point to neurovascular unit damage as a possible pathology associated with HAND in the absence of HIVE (Gelman et al. 2012; Wolf et al. 2002). Disturbances in bioenergetics and in glutamate homeostasis are reported in cART-treated HIV patients [see review (Saylor et al. 2016)]. Executive dysfunction, memory impairment with disruptions in attention, multi-tasking, impulse control, judgment and memory encoding, and retrieval and motor dysfunction are associated with HAND (Heaton et al. 2011; Saylor et al. 2016). Taking these findings into account, the iTat mouse model provides some but not all characteristics of patients with HAND and its use remains relevant to HAND studies.

Acknowledgments

Funding information This work was supported in part by the grants NIH/NINDS R01NS090960 and NIH/NIDA R01DA043162 (to JJH), and NIH/NIMH R01MH107340 and NIH/NIDA P01DA037830 (to DL and KK).

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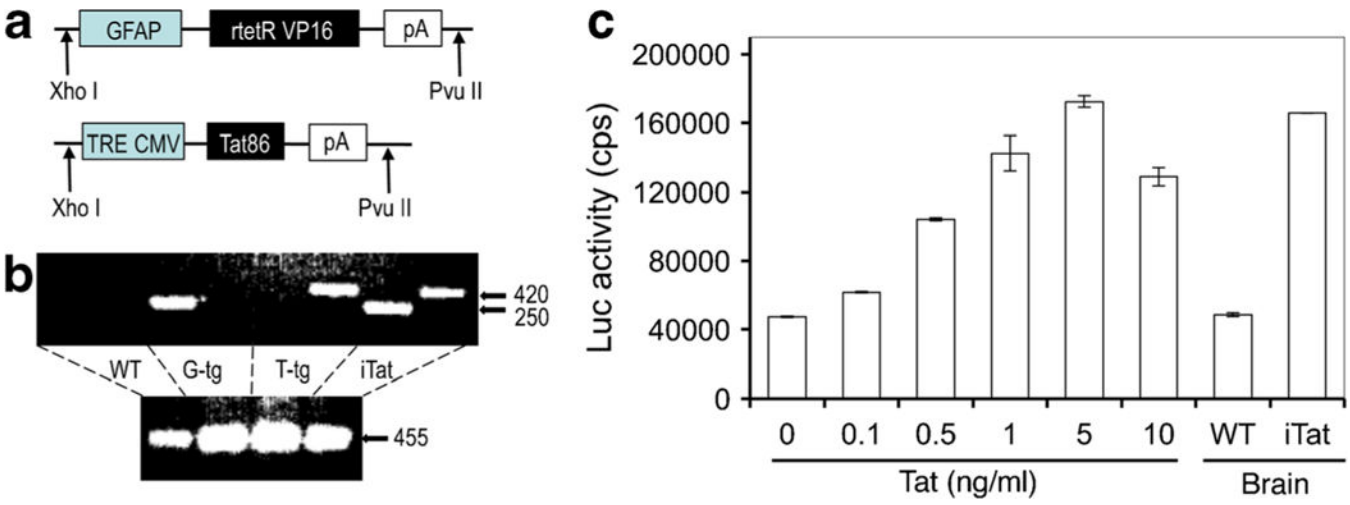

Fig. 1.

Generation of iTat mice and detection of Tat protein expression. **a** Two transgene constructs were constructed to make GFAP-rtTA transgenic mice (G-tg) and TRE-Tat86 transgenic mice (T-tg). **b** Genotyping of G-and T-tg mice and GT-tg (iTat) mice. Genomic DNA was isolated from the mouse tails and PCR was conducted using transgene-specific primers. GAPDH was included as a control. **c** Brain homogenates were prepared from Dox-treated iTat and wild-type (WT) mice and analyzed for the Tat protein using the LTR-driven luciferase reporter cell line TZM-bl and recombinant Tat standards. Modified from (Kim et al. 2003) with permission

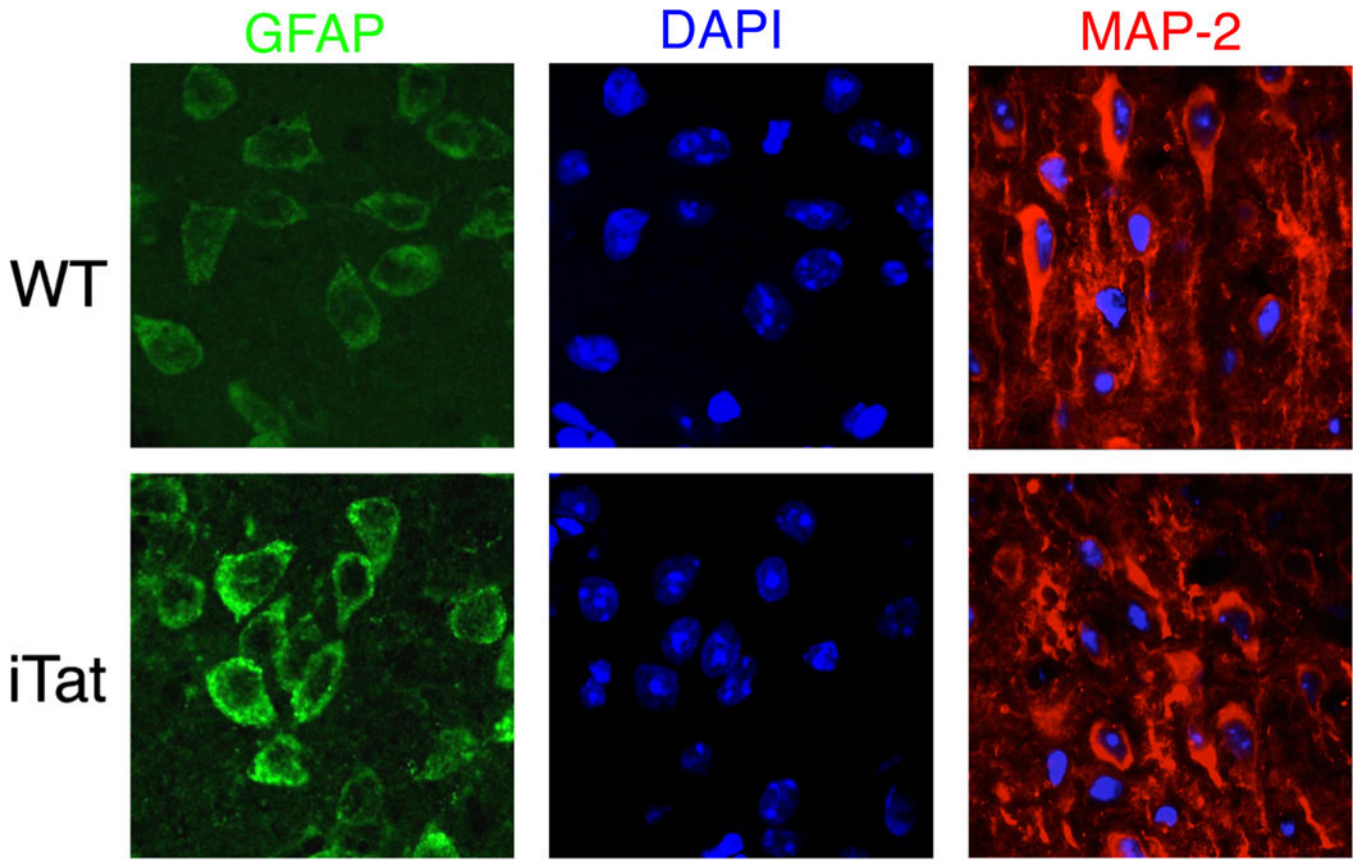

Fig. 2.

Increased GFAP expression and loss of neuronal dendrites in the brains of iTat mice. Wild-type (WT) and iTat mice were given Dox at 80 mg/kg/day for 7 days. The brains were collected for immunofluorescence labeling using anti-GFAP (green) and anti-MAP2 (red) antibodies. The sections were also counterstained with DAPI (blue)

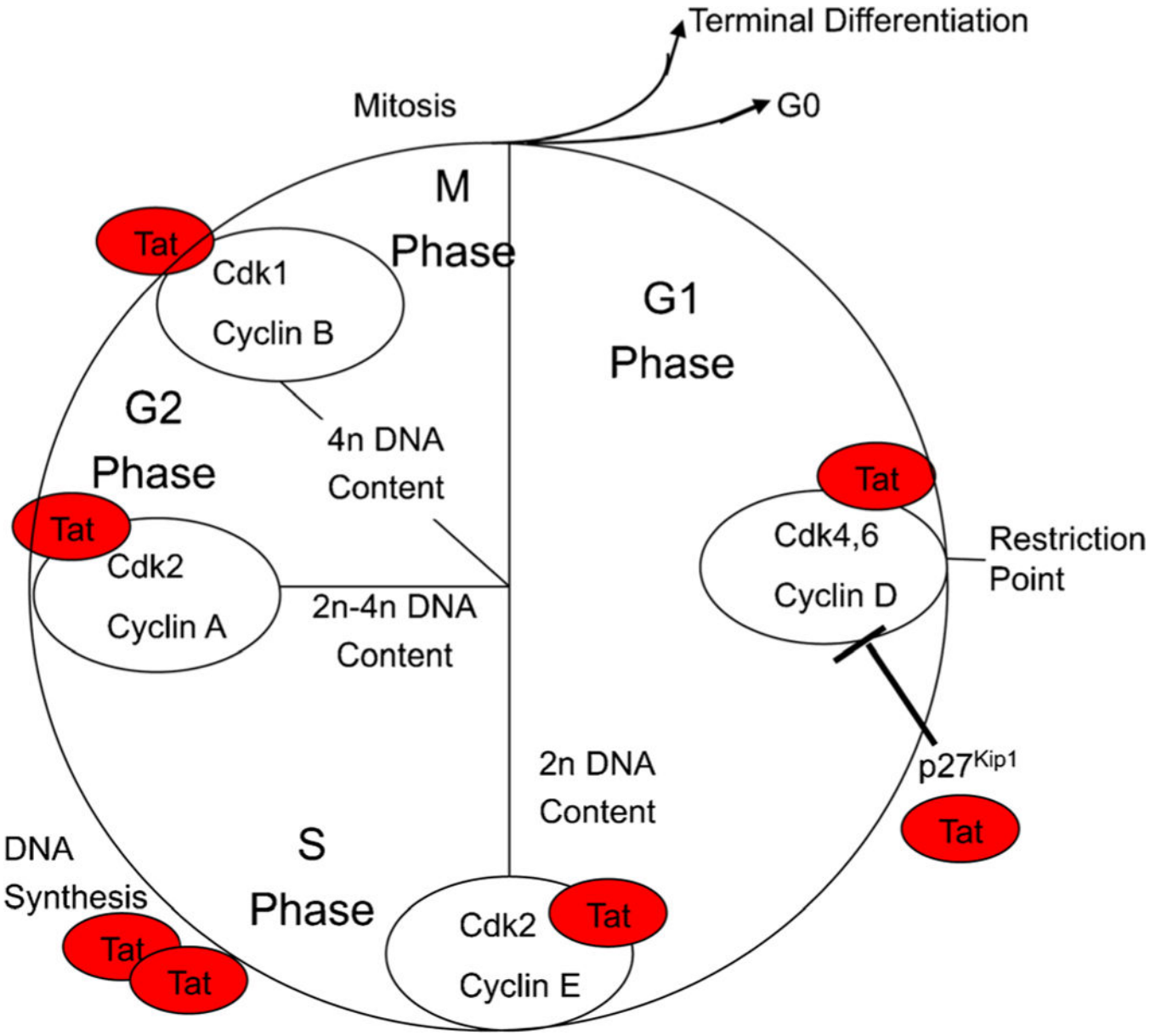

Fig. 3.

Interaction of Tat with cell cycle-related proteins. Cell lysates were prepared from Tat-expressing astrocytes and subjected to a protein-binding array analysis for cell cycle-related proteins. All Tat-binding proteins were identified as shown. Modified from (Zhou and He 2004) with permission

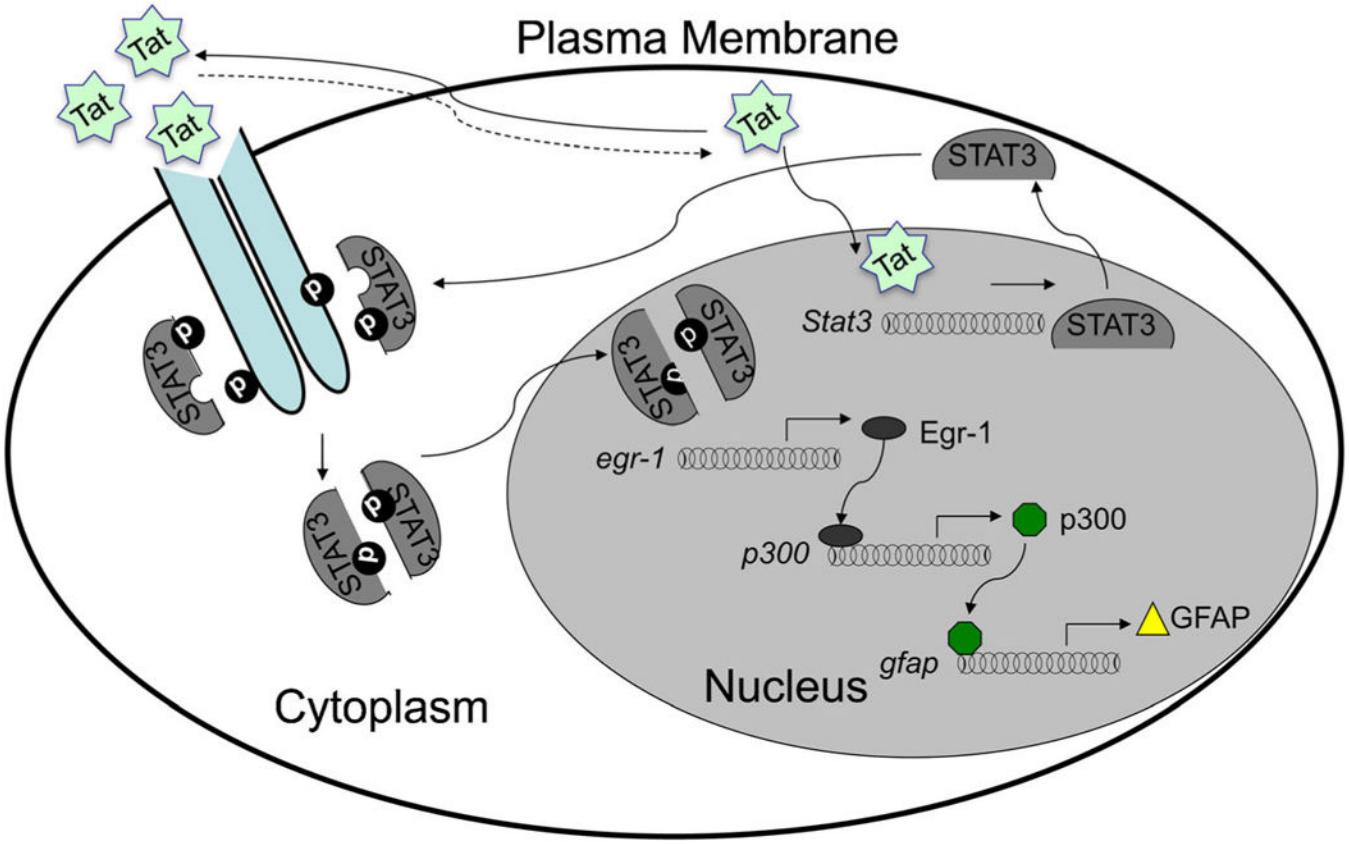

Fig. 4.

Transcriptional network for Tat-transactivated GFAP expression. Tat either transactivates STAT3 or interacts with cell surface receptors that lead to STAT3 phosphorylation and activation. Both routes result in sequential activation of Egr-1, p300 and GFAP. Modified from (Fan et al. 2015) with permission

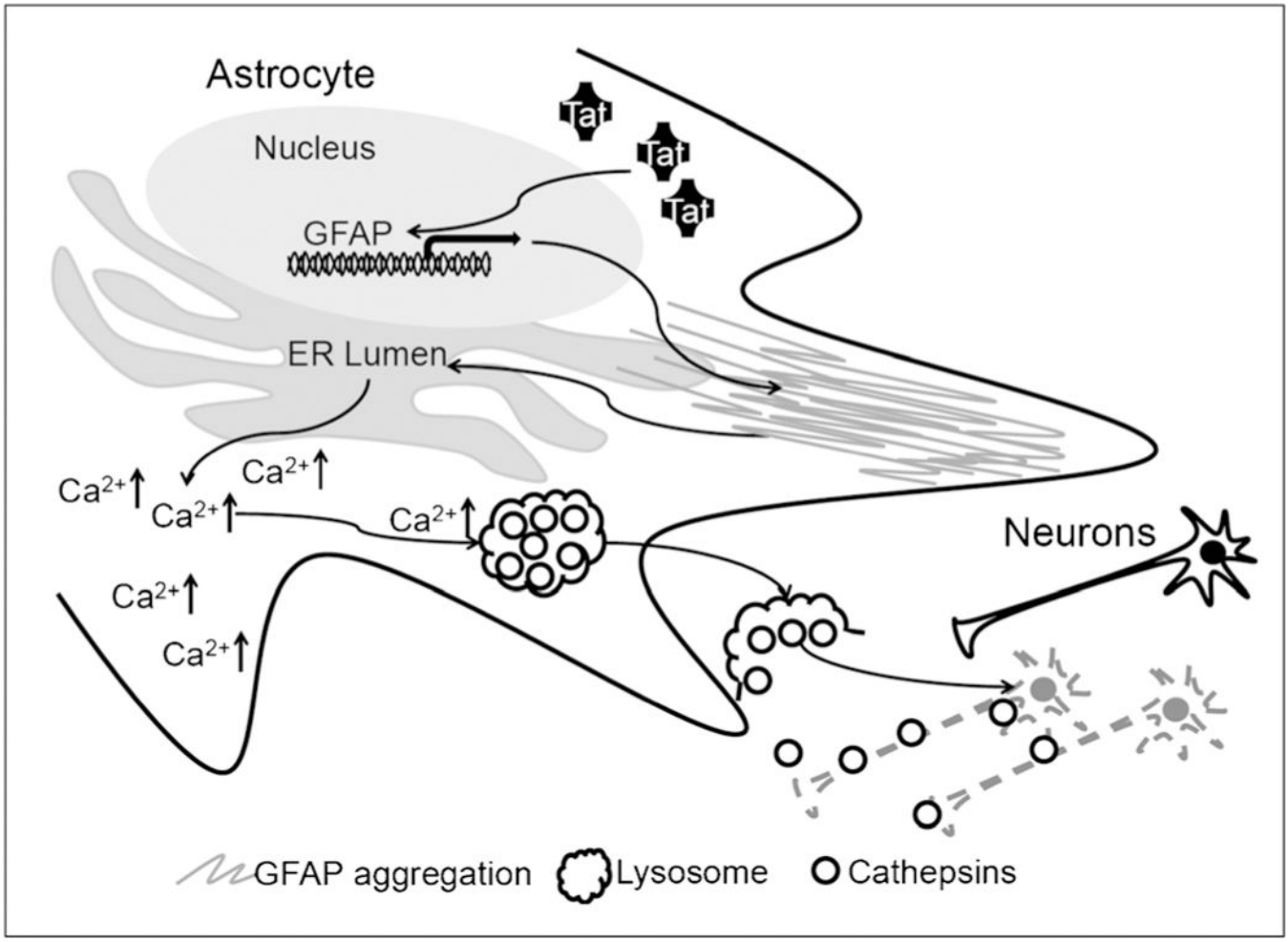

Fig. 5.

Astrocyte-mediated Tat neurotoxicity. Tat expression or uptake by astrocytes transactivates GFAP expression and causes GFAP aggregation and ER stress, which in turn triggers release of calcium from the ER and excessive lysosomal exocytosis and cathepsin-dependent neurotoxicity. Modified from (Fan and He 2016b) with permission

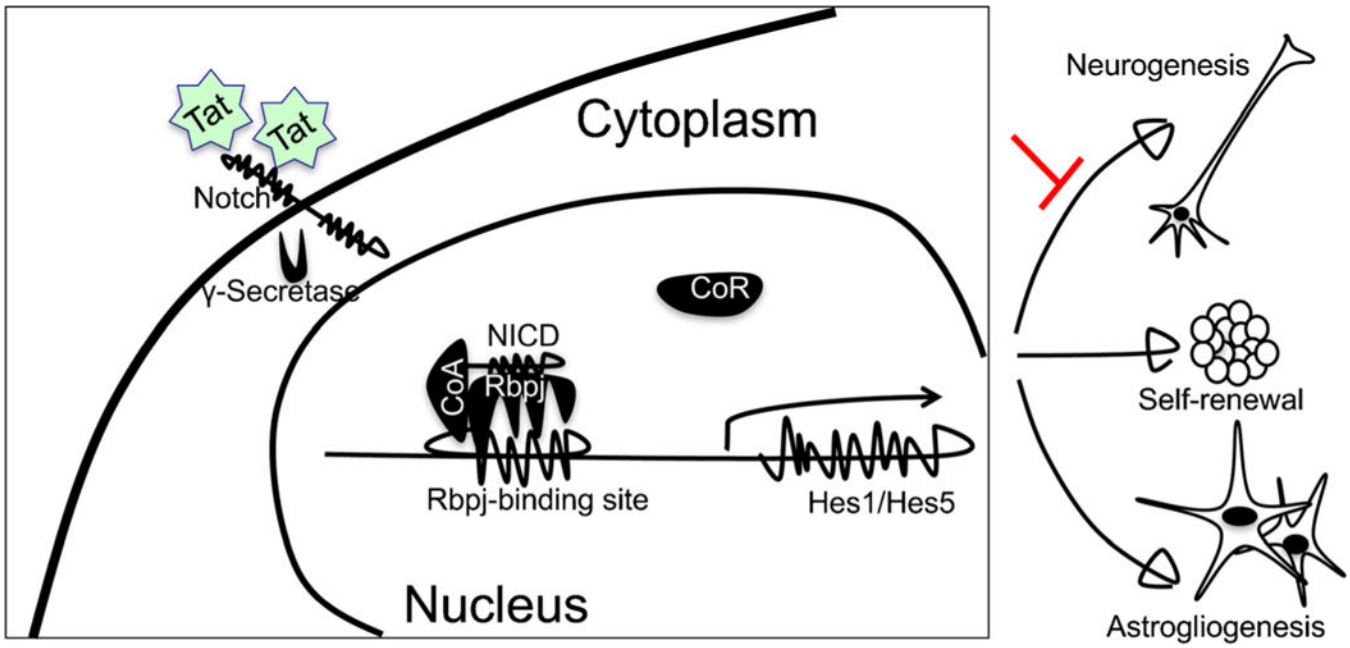

Fig. 6.

Tat promotes astrogliogenesis over neurogenesis through its binding to Notch to activate the Notch signaling pathway. Tat binds to the Notch extracellular domain and leads to cleavage of Notch intracellular domain (NICD) by γ-secretase. NICD translocates to the nucleus and binds to the Rbpj binding site on the Hes1 promoter, dissociates the co-repressor (CoR), and recruits the co-activator (CoA) to transactivate downstream molecular Hes1 expression. Activation of Notch signaling is sufficient to favor astrogliogenesis over neurogenesis

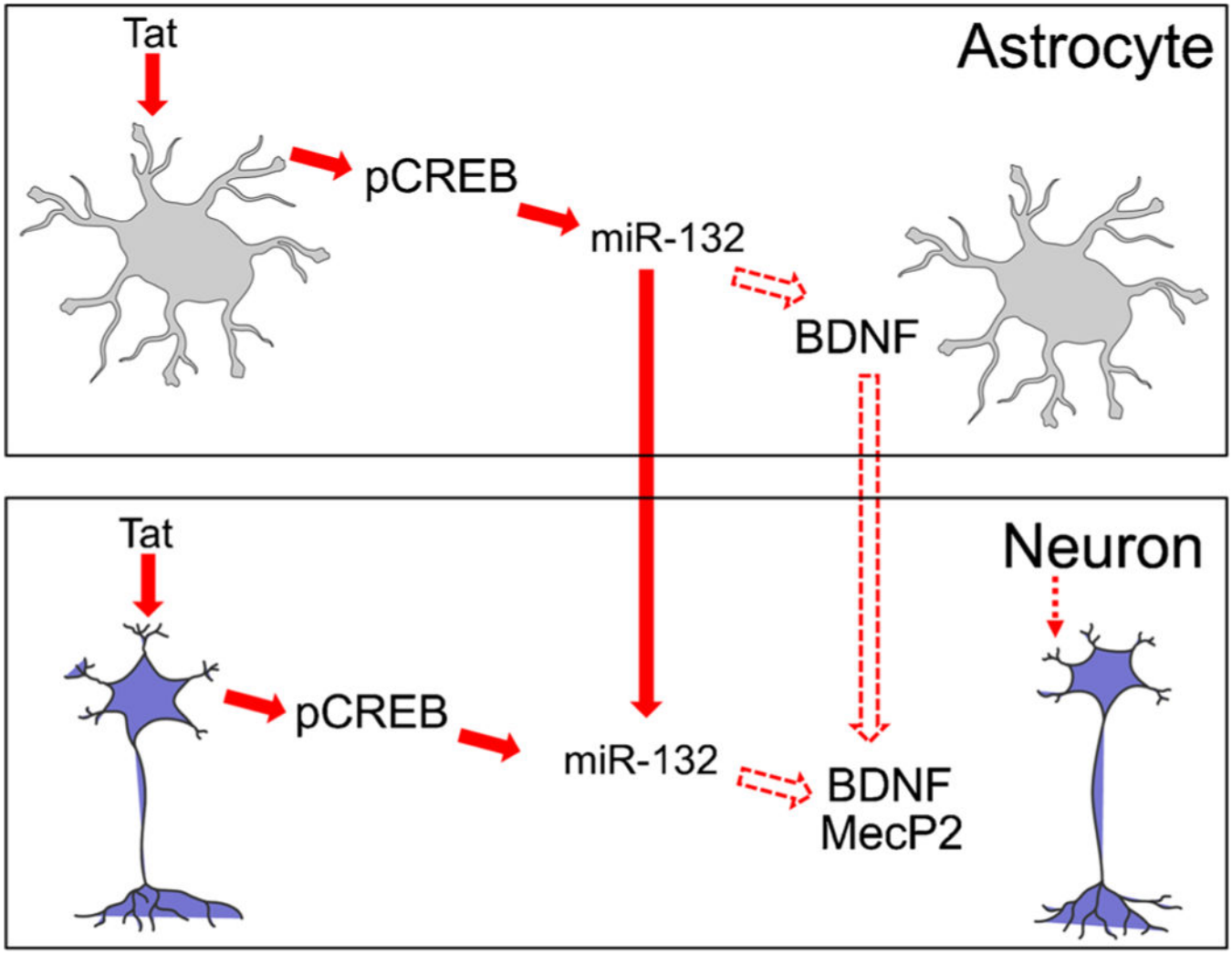

Fig. 7.

Tat, miR-132, and neurite growth. Tat expression leads to phosphorylation of CREB, which in turn transactivates miR-132 expression in both astrocytes and neurons. miR-132 down-regulates BDNF expression, while it is also taken up into neurons via exosomes and down-regulates MeCP2 expression in neurons. Both BDNF down-regulation in astrocytes and MeCP2 down-regulation in neurons contribute to Tat-induced neurite shortening. Reproduced from (Rahimian and He 2016b) with permission

Table 1

Use of iTat Mice in other studies

Mechanism(s) studied	References
Substance abuse	(Fitting et al. 2012, 2010; Hauser et al. 2009; Mediouni et al. 2015; Paris et al. 2014a; Zou et al. 2011)
Sex differences	(Hahn et al. 2015a, b)
Behavior	(Hahn et al. 2016; Paris et al. 2014b, c, 2015)
Learning and memory	(Carey et al. 2012; Fitting et al. 2013)
Structural changes	(Carey et al. 2015, 2013)
Lysosome/autophagosome	(Fan and He 2016b; Fields et al. 2015a)
Cell cycle	(Fields et al. 2015b)
Tau processing	(Kadri et al. 2015)
Enteric neuropathogenesis	(Ngwainmbi et al. 2014)
Cell survival/growth factors/differentiation	(Perry et al. 2010; Wheeler et al. 2008; Yao et al. 2012)
Glial activation	(Kiebala et al. 2010)
Microvascular changes	(Silva et al. 2014)