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Research report

# Proteomics analysis of human astrocytes expressing the HIV protein Tat

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**Abstract**

Astrocyte infection in HIV has been associated with rapid progression of dementia in a subset of HIV/AIDS patients. Astrogliosis and microglial activation are observed in areas of axonal and dendritic damage in HIVD. In HIV-infected astrocytes, the regulatory gene tat is over expressed and mRNA levels for Tat are elevated in brain extracts from individuals with HIV-1 dementia. Tat can be detected in HIV-infected astrocytes in vivo. The HIV-1 protein Tat transactivates viral and cellular gene expression, is actively secreted mainly from astrocytes, microglia and macrophages, into the extracellular environment, and is taken up by neighboring uninfected cells such as neurons. The HIV-1 protein Tat released from astrocytes reportedly produces trimming of neurites, mitochondrial dysfunction and cell death in neurons, while protecting its host, the astrocyte. We utilized proteomics to investigate protein expression changes in human astrocytes intracellularly expressing Tat (SVGA-Tat). By coupling 2D fingerprinting and identification of proteins by mass spectrometry, we identified phosphatase 2A, isocitrate dehydrogenase, nuclear ribonucleoprotein A1, Rho GDP dissociation inhibitor α, β-tubulin, crocalbin like protein/calumenin, and vimentin/α-tubulin to have decreased protein expression levels in SVGA-Tat cells compared to the SVGA-pcDNA cells. Heat shock protein 70, heme oxygenase-1, and inducible nitric oxide synthase were found to have increased protein expression in SVGA-Tat cells compared to controls by slotblot technique. These findings are discussed with reference to astrocytes serving as a reservoir for the HIV virus and how Tat promotes survival of the astrocytic host.


*Theme:* Disorders of the nervous system
*Topic:* Infectious diseases

*Keywords:* Proteomics; HIV; Tat; Astrocytes

## 1. Introduction

It is estimated that one-third of adults infected with human immunodeficiency virus (HIV-1) develop dementia (HIVD) [38]. HIV-1 dementia is now the leading cause of dementia in people younger than 60 years of age [55]. The pathological features associated with HIVD include microglial cell activation, astrocytosis, decreased synaptic and dendritic density, and selective neuronal loss [52]. In patients with HIV-1 infection, significant neuronal loss

*Abbreviations:* HIV, human immunodeficiency virus; HIVD, human immunodeficiency virus dementia; HIVE, human immunodeficiency virus encephalitis; AIDS, acquired immunodeficiency syndrome; AD, Alzheimer's disease; CSF, cerebral spinal fluid; BBB, blood–brain barrier; PP2A, protein phosphatase 2A; HnRNP, heterogenous nuclear ribonucleoprotein

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and dysfunction occur even though neurons are rarely infected [53,62]. The most commonly infected cell types in brain are microglia, macrophages and to some extent astrocytes, although limited viral replication is produced in astrocytes [44,46,53,62,70]. In cell cultures, HIV-1 infection of astrocytes results in an initial productive but non-cytopathogenic infection that diminishes to a viral persistence or latent state [56]. The major barrier to HIV-1 infection of primary astrocytes is at virus entry. Astrocytes have no intrinsic intracellular restriction to efficient HIV-1 replication [15]. Astrocytes may serve as a reservoir for the virus inducing neuronal damage by releasing cellular and viral products or loss of neuronal support functions.

In HIV-infected astrocytes, the regulatory gene *tat* is over expressed [60] and mRNA levels for Tat are elevated in brain extracts from individuals with HIV-1 dementia [95]. The HIV-1 protein Tat transactivates viral and cellular gene expression, is actively secreted into the extra cellular environment mainly from astrocytes, microglia and macrophages, and is taken up by neighboring uninfected cells such as neurons [19]. The Tat released from astrocytes reportedly produces trimming of neurites, mitochondrial dysfunction and cell death in neurons [19]. Conversely, Tat expressed intracellularly in stable astrocytic cell lines was able to protect astrocytes from cellular injury [19]. This finding demonstrates that Tat is a neurotoxin at distant sites, while protecting the environment where it is produced. Brain regions particularly susceptible to Tat toxicity include striatum [33], hippocampal dentate gyrus, and the CA3 region of the hippocampus [50]. HIV-infected astrocytes likely play a critical role in the neuropathogenesis of HIV infection and Tat has been hypothesized by many as a potential contributor to HIVD.

The mechanism in which Tat is capable of being present in astrocytes without causing cell death is unknown. In this study, we applied the method of proteomics to investigate changes in protein expression when Tat is expressed in human astrocytes, i.e., SVGA cells (SVGA-Tat), compared to SVGA cells expressing only the vector (SVGA-pcDNA). Proteins identified by proteomics provide insight into how astrocytes may act as a reservoir for the HIV virus.

## 2. Experimental procedures

### 2.1. Sample collection

SVGA Tat (SVGA-Tat) and vector (SVGA-pcDNA) expressing cells were constructed as previously described [19]. All cell lines were maintained in DMEM media with 5% fetal serum albumin and 1% antibiotic/antimycotic in an incubator at 37 °C and 5% $CO_2$. Cells were collected in isolation buffer containing protease inhibitors: 4 μg/mL leupeptin, 4 μg/mL pepstatin A, 5 μg/mL aprotinin, 20 μg/mL type II-s soybean trypsin inhibitor, 0.2 mM PMSF, 2 mM EDTA, 2 mM EGTA, 20 mM HEPES at pH 7.4, sonicated for 5 s to disrupt the cell membrane. Six sample preparations were collected and analyzed. Protein concentration was determined by the Pierce BCA method.

### 2.2. Two-dimensional gel electrophoresis

Two-dimensional polyacrylamide gel electrophoresis was performed in a Bio-Rad system using 110-mm pH 3–10 immobilized pH gradients (IPG) strips and Criterion 8–16% gels (Bio-Rad) following the method of Castegna et al. [17]. Samples were dissolved in two-dimensional polyacrylamide gel electrophoresis sample buffer [8 M urea, 2 M thiourea, 20 mM dithiothreitol, 0.2% (v/v) biolytes 3–10, 2% CHAPS, and bromophenol blue]. In the first-dimension, 200 μg of protein was applied to a rehydrated IPG strip, and the isoelectric focusing was carried out at 20 °C as follows: 300 V for 1 h, linear gradient to 800 V for 5 h and finally 20,000 V/h. Before the second dimensional separation, the gel strips were equilibrated for 10 min in 37.5 mM Tris–HCl (pH 8.8) containing 6 M urea, 2% (w/v) sodium dodecyl sulfate, 20% 9 v/v) glycerol, and 0.5% dithiothreitol, and then re-equilibrated for 10 min in the same buffer containing 4.5% iodoactamide in place of dithiothreitol. Strips were placed on Criterion gels and electrophoresis ran for 65 min at 200 V.

### 2.3. Image analysis

The gels were fixed in 10% methanol and 7% acetic acid for 30 min. Then stained with SYPRO Ruby Bio-Rad). Images from Sypro Ruby-stained gels were obtained using a STORM phosphoimager (ex. 470 nm, Em. 618 nm, Molecular Dynamics, Sunnyvale, CA, USA). The image intensity of the protein spots on the gels was quantified by HT Analyzer 2D PAGE software (Genomic Solutions).

### 2.4. Mass spectrometry

Samples were prepared using trypsin digestion techniques described by Jensen et al. [39], modified by Thongboonkerd et al. [86]. All mass spectra reported in this study were acquired from the University of Kentucky Mass Spectrometry Facility (UKMSF). A Bruker Autoflex MALDI TOF (matrix assisted laser desorption/ionization-time of flight) mass spectrometer (Bruker Daltonic, Billerica, MA, USA) operated in the reflectron mode was used to generate peptide mass fingerprints. Peptides resulting from in-gel digestion were analyzed on a 384-position, 600 μm Anchor-Chip™ Target (Bruker Daltonics, Bremen, Germany) and prepared according to AnchorChip recommendations (AnchorChip Technology, Rev. 2, Bruker Daltonics). Reported spectra are a summation of 100 laser shots. External calibration of the mass axis was used for acquisition and internal calibration using either trypsin autolysis ions or matrix clusters was applied post acquisition for accurate mass determination [17]. The mass spectra obtained were searched against the

NCBI protein databases using the MASCOT search engine (http://www.matrixscience.com).

### 2.5. HSP-70, HO-1 and iNOS detection

Protein concentration was determined by BCA. Six hundred nanograms of protein was blotted onto nitrocellulose paper by the slot blotting technique. Membranes were incubated with blocking buffer (PBS with 3% BSA, 0.2% Tween-20 and 0.01% sodium azide), for 30 min at room temperature, exposed to mouse anti-HSP-70, HO-1 or iNOS 1:1000 overnight followed by anti-mouse IgG coupled to alkaline phosphatase (1:10,000) for 1 h at room temperature. The blots were developed using SigmaFast Tablets™ and were analyzed using computer imaging software, Scion Imaging.

### 2.6. Statistical analysis

Statistical comparison of protein levels was analyzed by ANOVA. A value of $p<0.05$ was considered statistically significant.

## 3. Results

Protein expression level analysis by proteomics allows for identification of proteins with different expression levels in human astrocytic cells expressing Tat (SVGA-Tat) and SVGA cells expressing the vector (SVGA-pcDNA). Identification of possible proteins with significantly varying expression levels was achieved by mass spectrometry analysis. Protein spots from 2D gels used for mass spectrometry analysis were excised and subjected to trypsin digestion (Fig. 1). Six different sample preparations were analyzed. Table 1 presents the unique protein identifier (GI accession number), number of peptides identified, percent of sequence coverage, the probability-based MOWSE score and the $p$-value for each protein identified. Note that the latter is exceedingly small, indicating that the identity established for each protein is correct. Seven protein spots successfully identified by mass spectrometry analysis were found to have decreased expression levels in SVGA-Tat cells compared to SVGA-pcDNA cells (Table 2): heterogeneous nuclear ribonucleoprotein A1 helix-destabilizing protein (64±19%), isocitrate dehydrogenase 1 (67±9%),

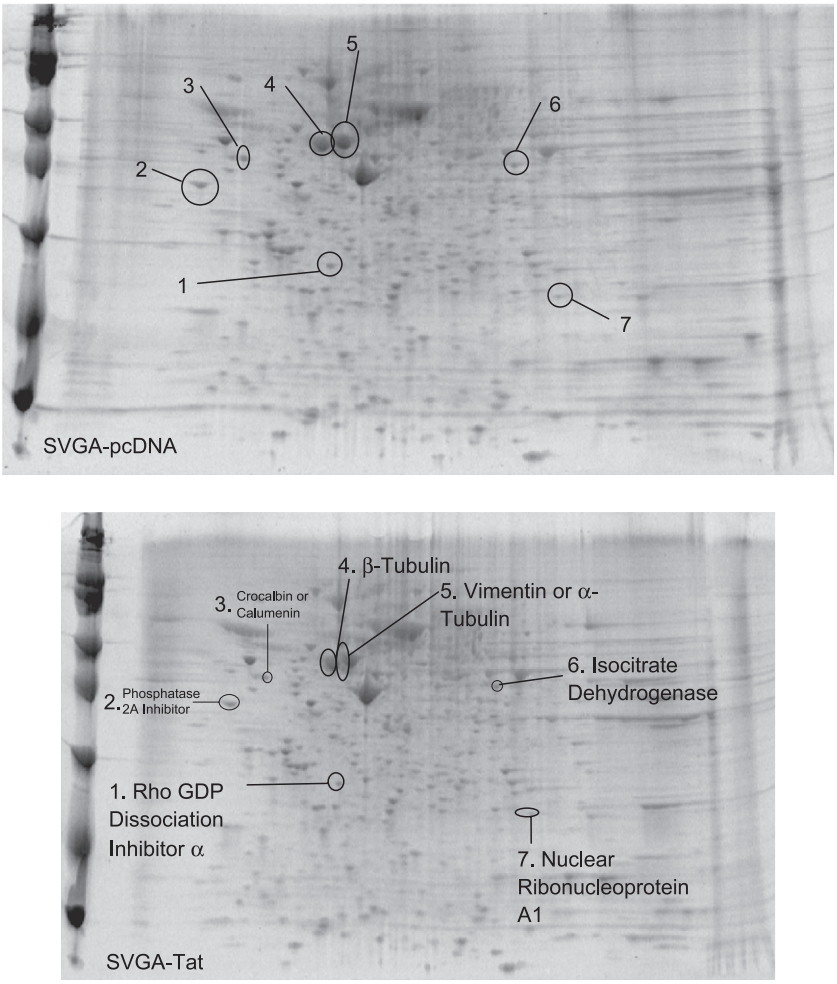


Fig. 1. 2D gel map of SVGA-Tat cells. Proteins identified by mass spectrometry are identified. The pH increases from left to right on the abscissa and the molecular mass decreases from top to bottom on the ordinate.

Table 1
Summary of proteins identified by mass spectrometry

| Identified protein | GI accession no. | No. of peptide matches identified | % Coverage matched peptides | p*I*, MrW (kDa) | Mowse score | Probability of a random hit |
|---|---|---|---|---|---|---|
| Crocalbin-like protein or calumenin | gi\|8515718 gi\|14718453 | 9 | 37 or 34 | 4.39, 34.97 or 4.41, 37.11 | 101 or 99 | $7.94\times10^{-11}$ or $1.25\times10^{-10}$ |
| Rho GDP dissociation inhibitor (GDI) alpha | gi\|4757768 | 6 | 30 | 5.02, 23.19 | 64 | $3.98\times10^{-7}$ |
| Beta-tubulin | gi\|2119276 | 11 | 27 | 4.71, 48.85 | 100 | $1\times10^{-10}$ |
| Protein phosphatase 2A (PP2A) inhibitor | gi\|1711383 | 8 | 34 | 4.12, 32.08 | 129 | $1.26\times10^{-13}$ |
| Heterogenous nuclear ribonucleoprotein A1 helix-destabilizing protein | gi\|133252 | 10 | 47 | 7.82, 22.12 | 136 | $2.51\times10^{-14}$ |
| Vimentin and alpha-tubulin 6 | gi\|2119204 gi\|14389309 | 12 10 | 37 32 | 5.06, 53.6 and 4.96, 53.7 | 183 for Mixture 101, 92 | $5.01\times10^{-19}$ (mixture) $7.94\times10^{-11}$ $6.31\times10^{-10}$ |
| Isocitrate dehydrogenase 1 ($NADP^+$) | gi\|5174471 | 12 | 24 | 6.19, 46.66 | 126 | $2.5\times10^{-13}$ |

Mowse scores greater than 63 are considered significant ($p<0.05$).

protein phosphatase 2A inhibitor (52±20%), β-tubulin (75±9%), and Rho GDP dissociation inhibitor (GDI) alpha (56±22%). Crocalbain was identified together with calumenin; these two proteins have the same isoelectric point (p*I*) and similar molecular mass with 88% amino acid homology. Vimentin was identified together with α-tubulin protein; these two proteins have the same p*I* and same molecular mass making it impossible to separate them by 2D PAGE. The protein expression level for the mixture of crocalbain and calumenin was 56±15% and vimentin and α-tubulin protein was 54±22% of the control spot. Typically in neurological diseases, protein expression levels identified by proteomics have changes of one to two fold differences [49]. For example, DRP-2 has about a 50% decrease protein expression level in Down Syndrome and Alzheimer's disease brain [49].

Several proteins were identified by slotblot and cell fluorescence techniques (data not shown), but not by proteomics. Heat shock protein 70, heme oxygenase 1, and inducible nitric oxide synthase were found to have significantly increased protein expression levels in SVGA-Tat cells compared the controls, SVGA-pcDNA (Figs. 2 and 3).

Table 2
Proteins expressed differently in SVGA-Tat cells compared to SVGA-pcDNA cells

| Identified proteins | Protein levels of SVGA-Tat cells[a] | *p*-value |
|---|---|---|
| Crocalbin-like protein or calumenin | 56±15% | $p<0.001$ |
| Rho GDP dissociation inhibitor (GDI) alpha | 56±22% | $p<0.015$ |
| Isocitrate dehydrogenase | 67±9% | $p<0.001$ |
| Beta-tubulin | 75±9% | $p<0.027$ |
| Protein phosphatase 2A (PP2A) inhibitor | 52±20% | $p<0.003$ |
| Vimentin and alpha-tubulin 6 | 54±22% | $p<0.004$ |
| Heterogenous nuclear ribonucleoprotein A1 helix-destabilizing protein | 64±19% | $p<0.013$ |
| Heat shock protein 70 (HSP70) | 136±0.05% | $p<0.001$ |
| Heme oxygenase-1 (HO-1) | 196±0.06% | $p<0.001$ |
| iNOS | 137±0.06% | $p<0.002$ |

[a] Percent of the level found in control SVGA-pcDNA cells if control is considered 100%, $n=6$.

## 4. Discussion

Significant neuronal loss and dysfunction occurs in HIV dementia even though neurons are rarely infected. Astrocytes may serve as a reservoir for the virus inducing neuronal damage by releasing cellular and viral products or loss of neuronal support functions. We recently demonstrated that Tat expressed intracellularly in astrocytes was not toxic to these cells [19]. In fact, intracellular Tat protected astrocytes from oxidative insults while other cells types expressing Tat experienced cell death [19]. In the current proteomics study, we found phosphatase 2A inhibitor, the mitochondrial enzyme isocitrate dehydrogenase, heterogenous nuclear ribonucleoprotein A1 helix-destabilizing protein, crocalbin/

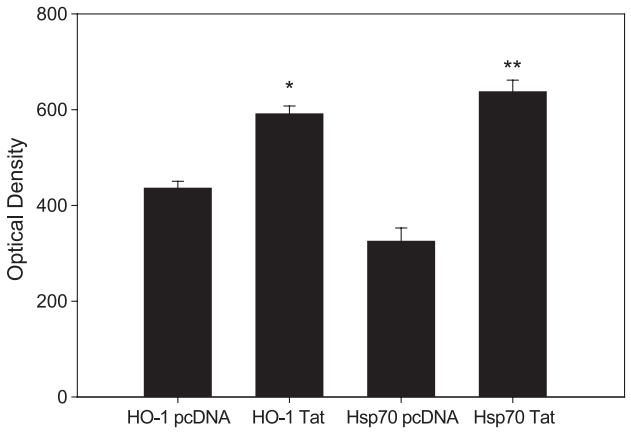


Fig. 2. Heat shock protein 70 and heme oxygenase-1 protein expression levels are significantly increased in SVGA-Tat cells compared to SVGA-pcDNA control cells ($n=4$, $p<0.01$).

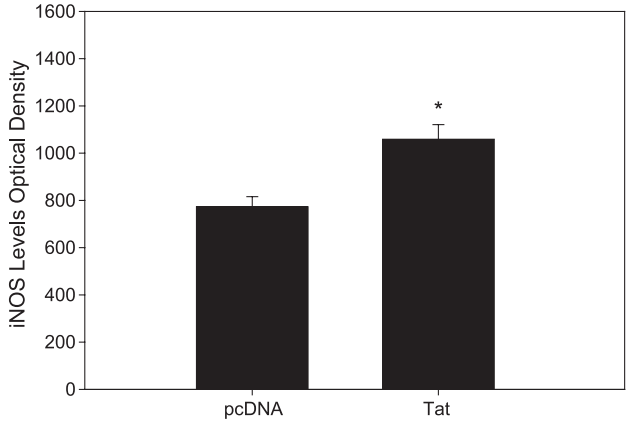


Fig. 3. iNOS protein expression levels are significantly increased in SVGA-Tat cells compared to SVGA-pcDNA control cells ($n$=4, $p$<0.01).

calumenin, β-tubulin, α-tubulin/vimentin, and Rho GDP dissociation inhibitor alpha to have decreased protein expression in SVGA-Tat cells compared to controls by proteomics. Heme oxygenase 1, heat shock protein 70 and iNOS were found to have increased protein expression by slotblot.

HIV-1 and gp120 have been demonstrated to either up- or down-regulate gene expression [27,81–83]. Transcripts regulated by HIV-1 in astrocytes code for G-coupled protein receptors, DNA binding, transcription factor, actin binding, and calcium binding, and many others had changes in gene expression [92] that parallel changes we found in protein expression. Several of the gene products detected in differential gene expression screens in infected astrocytes in culture could affect neuronal functions through a signaling network with astrocytes [8].

Protein phosphatase 2A (PP2A) is a phosphoprotein that plays important roles in the regulation of signal transduction, cell growth, and accounts for the majority of the Ser/Thr intracellular phosphatase activity. PP2A exists as a holoenzyme with three subunits A, B, and C or an AC heterodimer known as the core enzyme. The A subunit acts as a scaffold to which the B and catalytic C subunits bind. Many reports evidence that PP2A might play a role in regulating HIV-1 transcription and virus replication [28,69,85,87]. Ruediger et al. [69] demonstrated that increasing the ratio of PP2A core enzyme to holoenzyme resulted in a decrease in Tat-mediated HIV-1 transcription and virus production. Recently, it has been demonstrated that the catalytic subunit of PP2A (PP2Ac) is able to increase the basal activity of the HIV-1 promoter and markedly enhances the promoter's response to another stimulatory promoter agent PMA [25]. PP2Ac and PMA are shown to act synergistically to increase the level of proviral activation. Okadaic acid and fostriecin, potent inhibitors of PP2A, are able to reduce PMA activation of the HIV-1 enhancer and decrease HIV-1 proviral activation [25]. The decreased protein expression levels of PP2A inhibitor in human astrocytic cells expressing Tat shown in the current study is consistent with the notion of the possibility for continued HIV-1 transcription or inhibition depending on how the PP2A inhibitor interacts with the subunits of PP2A. Our data suggest that PP2A and its inhibitor are manipulated by Tat in astrocytes and may play a role in Tat-mediated HIV-1 transcription and virus production.

Rho is a family of small GTP-binding proteins. The Rho-family specific GDP-dissociation inhibitor, RhoGDI, forms complexes with Rho proteins in the cytosol of mammalian cells. RhoGDI stabilizes the inactive GDP-bound form of GTPases, preventing the release of GDP and the binding of GTP. CCR5 is a G protein-coupled receptor that binds several natural chemokines, but is also a coreceptor for the entry of M tropic strains of HIV-1 into cells. Levels of the chemokine receptor, CCR5, on the cell surface are important for the rate of HIV-1 infection and are determined by a number of factors including the rates of CCR5 internalization and recycling. Data indicate that CCR5 internalization and recycling are regulated by actin polymerization and activation of small G proteins in a Rho-dependent manner [59]. Accumulating evidence suggests Rho plays a role in tight junction disassembly in brain endothelial cells, in the blood–brain barrier (BBB), through activation of signaling pathways that regulate cytoskeletal organization [63,64,71]. Astrocytes are a significant component of the BBB, ensuring maintenance of brain microvascular endothelial cells and tight junctions, which are both altered in HIVE [21,65]. Rho GDP dissociation inhibitor alpha was found to be decreased in SVGA expressing Tat cells. These data suggest that Tat may affect Rho GTP signaling pathways through manipulation of expression levels of Rho GDP dissociation inhibitor, possibly allowing for increased signaling activation.

The cell cytoskeleton consists of microtubules and microfilaments and participates in processes that require changes in the shape of the cell and transport of materials from the nucleus to the synapse and back. There are three interconnected filament systems in eukaryotic cells: microfilaments consisting of actin, microtubules made from α/β-tubulin subunits, and intermediate filaments made from fibrous proteins such as vimentin. The HIV virus uses the host cytoskeletal system for infection and replication.

Proteases of retroviruses not only cleave viral polyproteins into their mature structural proteins and replication enzymes, but also cleave host cell proteins. HIV-1 and HIV-2 are capable of cleaving vimentin [76]. HIV-1 protease cleavage of vimentin is necessary for the changes in nuclear organization and chromatin distribution seen in HIV-1 [73]. The HIV-1 viral infectivity factor (*vif*) gene product is required for HIV-1 replication. It has been demonstrated that Vif localized with vimentin in perinuclear aggregates of HeLa cells [41]. Several strains of Vif are found to localize in the cytoplasm and induce dramatic perinuclear aggregation of vimentin in COS-7 cells, but not microtubular protein tubulin [34]. The cytoskeleton is important for virion

assembly, and reverse transcription is dependent on an intact actin network [10,77,78]. The interaction between actin and viral particle formation appears to be mediated by Gag [58], which associates with Vif during viral assembly [74,75]. It is possible that the interaction of Vif with cytoskeletal components such as vimentin is required for viral assembly, proper formation of the reverse transcription complex, and infectivity of the cell. Human astrocytes are rarely infected with HIV, but are thought to be hosts of viral proteins. The decrease in protein expression of vimentin in SVGA-Tat expressing cells may prohibit cytoskeleton-dependent proper virion assembly and reverse transcription.

α- and β-Tubulin are known to form heterodimers and function as the building blocks of microtubules, along with actin. Tat binds αβ-tubulin dimer and polymerized microtubules in the cytoplasm of the cell. This interaction is crucial for Tat to activate a mitochondrial-dependent apoptotic pathway [20]. Tat binds tubulin and microtubules through a four amino acid subdomain of its evolutionary conserved core region, and this interaction alters microtubule dynamics preventing microtubule depolymerization [20]. The Tat-tubulin interaction also delays the polymerization of tubulin and induces a premature stop to microtubule-dependent cytoplasmic streaming [7]. HIV proteins such as gp120 and Rev appear to affect cytoskeleton organization either by inducing cellular ultrastructural changes and massive disruption of microtubules [18,23] or by depolymerizing microtubules via a specific Rev-tubulin interaction [93]. We report here a decreased protein expression of β-tubulin in SVGA cells expressing Tat compared to SVGA-pcDNA cells. Since the binding of Tat to β-tubulin is crucial for induction of mitochondrial dependent apoptotic pathways, decreased levels of β-tubulin in astrocytic cells may stabilize the cell allowing the astrocyte to serve as a host for the release of Tat and other viral proteins.

The first oxidative conversion in the TCA cycle is catalyzed by isocitrate dehydrogenase. Isocitrate and $NAD^+$ are converted to α-ketoglutarate, NADH and $CO_2$. Isocitrate dehydrogenase is a highly regulated enzyme. The enzyme is stimulated by $NAD^+$ and ADP and inhibited by NADH, making it very sensitive to the NADH-to-$NAD^+$ ratio. Rats administered zidovudine (AZT), an antiviral drug often taken by HIV patients, had a significant decrease in isocitrate dehydrogenase activity. We report here a decreased expression of isocitrate dehydrogenase in SVGA-Tat cells. We previously demonstrated that SVGA cells expressing Tat can protect their mitochondria from 3-nitroproprionic acid, a mitochondrial toxin [19].

The HIV-1 primary RNA transcript is alternatively spliced into more than 40 different mRNAs. Alteration of the complex splicing pattern generating the viral mRNAs can dramatically affect HIV-1 infectivity and pathogenesis [68,94]. The heterogenous nuclear ribonucleoprotein (hnRNP) family serve as host factors that bind to HIV-1 regulatory elements [45], and are often involved in negative regulation. The binding properties of hnRNP A1 to different nucleic acid substrates have been thoroughly investigated [45], and it has become evident that hnRNP A1 interacts with a broad range of RNA substrates. Five splicing inhibitory sequences in the HIV-1 pre-mRNA have been identified. ESS2 and ESS2p are located within *tat* exon 2 [5,37], and ESS3 is located within *tat* exon 3 [6,22,51,79,97]. It has been shown that hnRNPs of the A/B family bind ESS2, ESS3, ESSV, and ISS HIV elements to repress splicing of the upstream intron [9,16,84,97]. HnRNP A1 appears to bind cooperatively to ESS2, ESS3, a UAGNNAUG motif in the ESE and to an ISS [22,51,96]. We report here protein expression of hnRNP A1 to be decreased in human astrocytes expressing Tat. The data suggest that Tat down-regulates proteins such as hnRNP A1 that may suppress tat-regulated HIV-1 transcription.

Calumenin and crocalbin (CBP-50) are in the EF-hand motif family. These proteins are present throughout the entire secretory pathway and possess a low $Ca^{2+}$ affinity with dissociation constants in the mM level [88]. The only known function of crocalbin is as mediator of the snake venom crotoxin, which posses phospholipase A2 activity [35,36]. PLA2s play important roles in phospholipid metabolism, host defense, and signal transduction. Free fatty acids released, depending on their nature, can participate in inflammatory pathways. Calumenin can be found in the ER and Golgi complex as well as be secreted into the medium of cultured cells [89]. Calumenin has been shown to bind serum amyloid P component and in solution to form high molecular mass aggregates with about 10 or more molecules [90]. We identified spot 33 to have decreased protein expression in SVGA-Tat cells. Mass spectrometry analysis identified both calumenin and crocalbin as the possible protein of interest. Calumenin and crocalbin have similar molecular weight and p*I*'s with 88% amino acid homology. Due to structural similarities between calumenin and crocalbin they are likely to share some functional properties.

NO is a diffusible gas that plays an important role in many physiological and diverse pathophysiological conditions [48]. At low concentrations, NO plays a unique role in neurotransmission and vasodilation, whereas at higher concentrations it is neurotoxic [61]. The exposure of microglia to Tat led to dose-dependent expression of iNOS and NO production [66], and human astroglial cells expressing Tat induced NO production and expression of iNOS protein and mRNA [48]. We found that SVGA-Tat cells induce protein expression of iNOS confirming the above report (Fig. 3). iNOS correlates with severity of HIV-associated dementia [1,2], and addition of NO to HIV-1 infected cells produces a significant increase in viral replication, which is blocked by specific inhibitors of iNOS [29,40]. NO derived in excessive amounts from the activation of inducible nitric-oxide synthase in glial cells and macrophages is assumed to contribute to neuronal abnormalities in HIVD.

Cells are known to induce heat shock proteins (Hsps), particularly HSP-70 and HSP-72, upon injury, including hypoxia ischemia, seizure, heat, epilepsy, trauma, neurodegenerative disorders, metabolic disorders, viral, bacterial and parasitic infection, inflammation, oxidant injury and heavy metals [14,67]. A number of studies have shown that Hsps protect CNS cells against many types of insults. Hsps can also protect cells from the consequences of protein misfolding and induce anti-apoptotic proteins, such as bcl-2 [42]. The normal function of HSP-70 is that of a molecular chaperone of proteins for translocation into different cellular compartments. HSP-70 can interact directly with fatty acids and may be part of their mode of binding to cell membranes [30,31]. HSP70 has been shown to play an important role in the life cycle of different RNA and DNA viruses [72]. Acute HIV infection leads to increase mRNA and protein level of HSP70 [91], redistribution of HSP70 on the cell surface of the infected cells [24], and HSP70 may be involved in the prevention of HIV-induced apoptosis [57]. HSP70 is also thought to play a role in the nuclear import of HIV-1 complexes [4]. The HIV-1 gag protein interacts with HSP70 during formation and release of the HIV-1 virion from the infected cells [32]. HSP70 and other Hsps incorporate into the membrane of HIV virions, which may lead to an increased immune response. Increased levels of anti-HSP70 antibodies have been measured in the sera and lymphocytes of patients with HIV infection [3,43]. Although, gp120 was unable to induce expression of Hsp70, over expression of Hsp70 allowed complete protection against gp120 insult [47]. Here, we demonstrated that SVGA cells expressing Tat have increased protein expression levels of HSP70 compared to controls. The data suggest that Tat is responsible for inducing HSP70 and that HSP70 may play a role in protecting astrocytes from cell death induced by HIV proteins. Cells treated with nitric oxide generating compounds demonstrated increased HSP70 protein expression, thus suggesting a role for NO in inducing HSP70 [11].

Heme oxygenase 1 (HO-1) also known as HSP32, is an inducible stress protein that has been implicated in defense mechanisms against agents that may induce oxidative injury such as endotoxins, cytokines and heme [13]. HO catalyzes the rate-controlling step of heme degradation into CO, iron, and the antioxidant biliverdin. The latter is converted rapidly by the enzyme biliverdin reductase to bilirubin, a potent antioxidant at low levels [12,67]. HO-1 is mainly synthesized in microglia. Here, we found increased protein expression levels of HO-1 in SVGA cells expressing Tat. HO-1 may increase levels of antioxidants by degrading heme to produce the known antioxidant biliverdin and subsequently bilirubin. The availability of sufficient heme is essential for the formation of functional iNOS dimmers and catalytic activity [80]. Thus, HO-1-mediated heme degradation may be a negative feedback regulation for iNOS-derived NO production [26], thereby protecting cells from NO-induced oxidative stress.

The human immunodeficiency virus (HIV)-Tat protein has been implicated in the neuropathogenesis of HIV infection. However, its role in modulating astroglial–neuronal relationships is poorly understood. Astrocyte infection with HIV has been associated with rapid progression of dementia. Astrogliosis and microglial activation are observed in areas of axonal and dendritic damage in HIVD [54]. The irony is that while HIV-released proteins (e.g., Tat; gp120), are toxic to neurons that are not infected by the virus, astrocytes, loaded with HIV are protected. This proteomics study has identified expression levels of proteins whose functions may partially explain the survivability of HIV-infected astrocytes. The proteins identified as differently expressed in SVGA-Tat cells, are involved in metabolism (isocitrate dehydrogenase), cell signaling (PP2A inhibitor, Rho GDP dissociation inhibitor, iNOS), axonal structure (β-tubulin, α-tubulin, vimentin), and cytoprotective measures (HSP70, HO-1, crocalbin, calumenin). These proteins may be critical in understanding how HIV utilizes astrocytes as hosts, without destruction of the host. Further studies will be needed to investigate the relationships of these proteomic-identified proteins and the role of HIV infected astrocytes in HIV dementia.

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