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Research report

Proteomic analysis of oxidatively modified proteins induced by the mitochondrial toxin 3-nitropropionic acid in human astrocytes expressing the HIV protein tat

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Abstract

The human immunodeficiency virus (HIV)-Tat protein has been implicated in the neuropathogenesis of HIV infection. However, its role in modulating astroglial function is poorly understood. Astrocyte infection with HIV has been associated with rapid progression of dementia. Intracellularly expressed Tat is not toxic to astrocytes. In fact, intracellularly expressed Tat offers protection against oxidative stress-related toxins such as the mitochondrial toxin 3-nitroproprionic acid (3-NP). In the current study, human astrocytes expressing Tat (SVGA-Tat) and vector controls (SVGA-pcDNA) were each treated with the irreversible mitochondrial complex II inhibitor 3-NP. Proteomics analysis was utilized to identify changes in protein expression levels. By coupling 2D fingerprinting and identification of proteins by mass spectrometry, actin, heat shock protein 90, and mitochondrial single-stranded DNA binding protein were identified as proteins with increased expression, while lactate dehydrogenase had decreased protein expression levels in SVGA-Tat cells treated with 3-NP compared to SVGA-pcDNA cells treated with 3-NP. Oxidative damage can lead to several events including loss in specific protein function, abnormal protein clearance, depletion of the cellular redox-balance and interference with the cell cycle, ultimately leading to neuronal death. Identification of specific proteins protected from oxidation is a crucial step in understanding the interaction of Tat with astrocytes. In the current study, proteomics also was used to identify proteins that were specifically oxidized in SVGA-pcDNA cells treated with 3-NP compared to SVGA-Tat cells treated with 3-NP. We found β-actin, calreticulin precursor protein, and synovial sarcoma X breakpoint 5 isoform A to have increased oxidation in control SVGA-pcDNA cells treated with 3-NP compared to SVGA-Tat cells treated with 3-NP. These results are discussed with reference to potential involvement of these proteins in HIV dementia and protection of astrocytes against oxidative stress by the HIV virus, a prerequisite for survival of a viral host cell.

Theme: Disorders of the nervous system
Topic: Infectious diseases

Keywords: Proteomics; HIV; Tat; Astrocytes; Protein oxidation

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; 3-NP, 3-nitroproprionic acid; mtSSB, mitochondrial single-stranded DNA-binding protein; LDH, lactate dehydrogenase

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1. Introduction

It is estimated that one-third of adults infected with human immunodeficiency virus (HIV-1) develop dementia (HIVD) [19]. The pathological features associated with human immunodeficiency virus dementia (HIVD) include microglial cell activation, astrocytosis, decreased synaptic and dendritic density, and selective neuronal loss [30]. In patients with HIV-1 infection, significant neuronal loss and dysfunction occur even though neurons are rarely infected [31,38]. The most commonly infected cell types in brain are microglia, macrophages and to some extent astrocytes, although limited viral replication is produced in astrocytes [22,23,31,38,46]. 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 [33]. 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 [6]. 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 [36] and mRNA levels for Tat are elevated in brain extracts from individuals with HIV-1 dementia [57]. 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 [11]. The HIV-1 protein Tat released from astrocytes reportedly produces trimming of neurites, mitochondrial dysfunction and cell death in neurons [11]. Intracellular Tat is not toxic to astrocytes. In fact, Tat produced in astrocytic cell lines was able to protect astrocytes from cellular injury induced by 3-nitropropionic acid (3-NP), a mitochondrial toxin; whereas, HeLa cells expressing Tat were not protected [11]. This finding demonstrates that Tat is a neurotoxin at distant sites while protecting the environment where it is produced.

The mechanism in which Tat is capable of protecting astrocytes from cellular death is unknown. In this study, we applied proteomics to identify proteins that change expression levels and proteins that are protected from oxidation when Tat is expressed in human astrocytes, (SVGA-Tat) cells, compared to SVGA cells expressing only the vector (SVGA-pcDNA), after both were treated with the mitochondrial toxin and oxidative stress inducer 3-nitropropionic acid. 3-NP is used since it is known to cause mitochondrial dysfunction and oxidative stress in the brain [24,25], and Tat exposed to neurons results in mitochondrial dysfunction and oxidative stress [11,43]. The proteomics-identified proteins provide insight into potential mechanisms by which astrocytes may act as a reservoir for the HIV virus.

2. Experimental procedures

2.1. Sample collection

SVGA-Tat- and vector (SVGA-pcDNA)-expressing cells were constructed as previously described [11]. 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 different sample preparations were collected and analyzed. Protein concentration was determined by the Pierce BCA method.

2.2. Two-dimensional gel electrophoresis

Cell-derived proteins (200 μg) were incubated with 4 volumes of 2 N HCl for electrophoresis or incubated with 4 volumes of 20 mM DNPH for Western blotting, in both cases at room temperature (25 °C) for 20 min followed by TCA precipitation and three washings with 1:1 (v/v) ethanol/ethyl acetate solution. 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. [8]. 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. SYPRO ruby staining

The gels were fixed in 10% methanal and 7% acetic acid for 30 min. Then stained with SYPRO Ruby gel stain (Bio-Rad). The SYPRO Ruby stain was then removed and gels were stored in dI water.

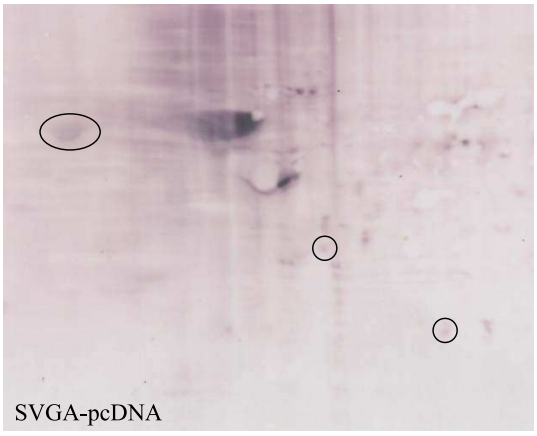
2.4. Western blotting

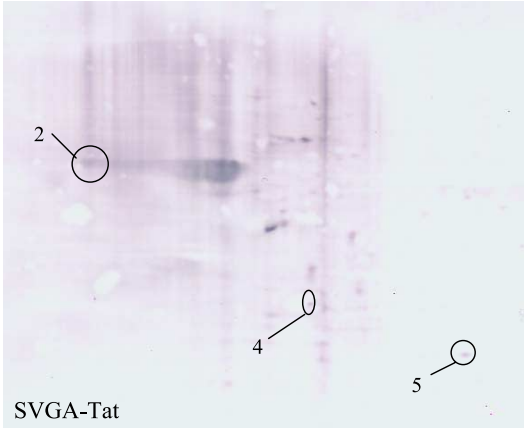
The gels were prepared in the same manner as for 2D-electrophoresis. After the second dimension, the proteins from gels were transferred to nitrocellulose papers (Bio-

Rad) using the Transblot-Blot® SD semi-Dry Transfer Cell (Bio-Rad) at 15 V for 4 h. The 2,4-dinitrophenyl hydrazone (DNP) adduct of the carbonyls of the proteins was detected on the nitrocellulose paper using a primary rabbit antibody (Intergen) specific for DNP–protein adducts (1:100), and then a secondary goat anti-rabbit IgG alkaline phosphatase (Sigma) antibody was applied. The resultant stain was developed by application of Sigma-Fast (BCIP/NBT) tablets.

2.5. Image analysis

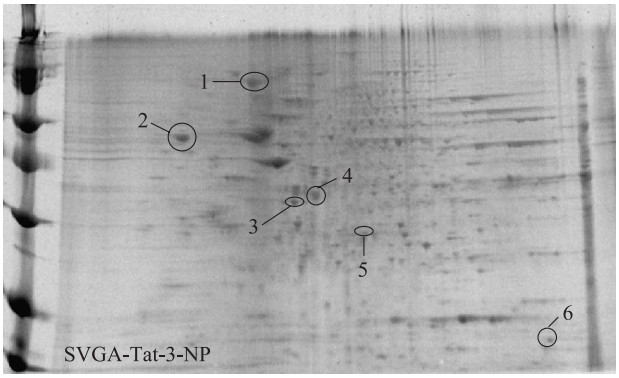
Images from Sypro Ruby stained gels were obtained using a STORM phosphoimager (ex. 470 nm, Em. 618 nm, Molecular Dynamics, Sunnyvale, CA, USA). The Western blots were scanned and saved in TIFF format using a Scanjet 3300 C (Hewlett Packard). The image intensity of the protein spots on the gels and Western blots were then quantified by PD-Quest (Bio-Rad).

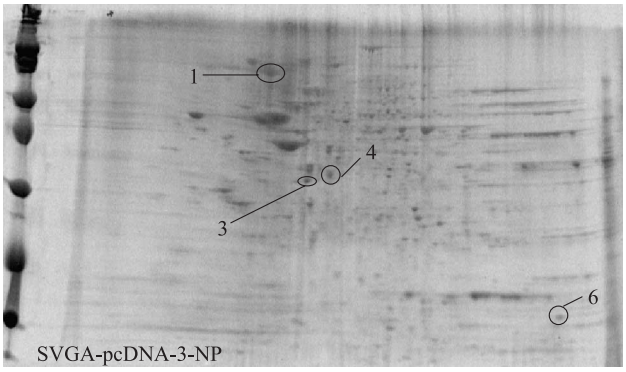




Corresponding Proteins
2 Calreticulin Precursor Protein
4 Beta-Actin
5 Synovial Sarcoma X Breakpoint 5 Isoform A

Fig. 1. Oxyblot maps for SVGA-pcDNA cells treated with 3-NP, control, (top) and SVGA-Tat cells treated with 3-NP (bottom) labeled with the proteins less oxidized in SVGA-Tat cells treated with 3-NP. Protein oxidation is normalized to specific protein concentration. The numbering system corresponds to that of Fig. 2.





Corresponding Proteins
1 Heat Shock Protein 90
2 Calreticulin Precursor Protein
3 Lactate Dehydrogenase
4 Beta-Actin
5 Synovial Sarcoma x Breakpoint 5 Isoform A
6 Mitochondrial Single-Stranded DNA Binding Protein

Fig. 2. 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. Molecular weight markers are on the left of the gel.

2.6. Mass spectrometry

Samples were prepared using trypsin digestion techniques described by Jensen [20], modified by Thongboonkerd et al. [53]. 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 [8]. The mass spectra obtained were searched against the NCBI protein

Table 1
Proteomics characteristics of proteins identified by mass spectrometry in SVGA-Tat cells treated with 3-NP compared to SVGA-pcDNA treated with 3-NP

Identified protein	GI accession numbers	No. of peptide matches identified	% Coverage matched peptides	p*I*, MrW	Mowse score	Probability of a random hit
β-Actin	gi|14250401	6	23	5.55, 41	68	1.58×10^{-7}
Heat shock protein 90	gi|20149594	18	30	4.97, 83.2	162	6.3×10^{-17}
Mitochondrial single-stranded DNA binding protein	gi|2624694	6	27	8.23, 15.2	86	2.5×10^{-9}
Lactate dehydrogenase	gi|13786847	8	34	5.72, 36.5	66	2.5×10^{-7}
Calreticulin precursor protein	gi|4757900	8	47	4.29, 47.6	99	1.3×10^{-10}
β-Actin	gi|14250401	5	15	5.56, 41	70	1×10^{-7}
Synovial sarcoma X breakpoint 5 isoform a	gi|16741685	6	17	9.61, 26	67	2×10^{-7}

Mowse scores greater than 63 are considered significant ($p<0.05$), $n=6$.

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

2.7. Statistics

The data of protein level and protein specific carbonyl level were analyzed by Student's *t*-tests. A value of $p<0.05$ was considered statistically significant.

3. Results

In the current study, we tested the hypothesis that inhibition of mitochondrial complex II by 3-NP, a process associated with oxidative stress in rat brain [24,25], in astrocytes expressing Tat would protect proteins from oxidation. We utilized proteomics to investigate the effect that 3-NP has on proteins in SVGA cells expressing the HIV protein Tat. Proteomic analysis permits identification of differences in protein expression levels and oxidation levels of proteins as measured by protein carbonyls. Comparison of 2D oxyblots (Fig. 1) with images of Sypro stained 2D gels (Fig. 2) from the same samples revealed that many, but not all, individual protein spots exhibited anti-protein carbonyl immunoreactivity. 2D oxyblots and the subsequent 2D gel images were matched and the anti-DNPH immunoreactivity of individual proteins separated by 2D PAGE was normalized to their protein content, obtained by measuring the intensity of Sypro Ruby staining [7,8,9].

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. Four out of eighteen proteins exhibiting differing levels of expression were identified by mass spectrometry. The remaining proteins were unable to be identified beyond statistical significance. We identified heat shock protein 90 (121±3.3% of control) and mitochondrial single-stranded DNA binding protein (113±4% of control) to have increased protein expression in SVGA-Tat cells treated with the mitochondrial toxin 3-NP (Table 2). Actin (64±1.9% of control) and lactate dehydro-

Table 2
Proteins expressed differently in SVGA-Tat cells compared to SVGA-pcDNA cells, both treated with 3-NP

Identified proteins	Protein levels of SVGA-Tat cells[a]	*p*-value
Actin	64±1.9%	$p<0.02$
Heat shock protein 90	121±3.3%	$p<0.002$
Mitochondrial single-stranded DNA binding protein	113±4%	$p<0.01$
Lactate dehydrogenase	51±2.6%	$p<0.007$

[a] Percent of the level found in control SVGA-pcDNA cells treated with 3-NP, taken to be 100%.

Table 3
Proteins with decreased oxidation as measured by protein carbonyl levels in SVGA-Tat cells compared to SVGA-pcDNA cells, both treated with the mitochondrial toxin, 3-NP

Identified proteins	Sequence of identified proteins	Percent decrease in protein carbonyl levels of proteins from SVGA-Tat cells treated with 3-NP[a]	*p*-value
β-Actin	DLTDYLMK+ Oxidation (M) GYSFTTTAER EITALAPSTMK+ Oxidation (M) QEYDESGPSIVHR SYELPDGQVITIGNER	81±2.3%	$p<0.04$
Calreticulin precursor protein	WIESKHK GLQTSQDAR VHVIFNYK DKQDEEQR FYGDEEKDK KVHVIFNYK EQFLDGDGWTSR FYALSASFEPFSNK	79±2.2%	$p<0.04$
Synovial sarcoma X breakpoint 5 isoform A	EWEKMK VGSQIPQK KYEAMTK GKHAWTHR ALLCGRGEAR LGFKATLPPFMR+ Oxidation (M)	74±1.6%	$p<0.02$

Oxidation (M): oxidation of methionine.
[a] Percent of the level found in SVGA-pcDNA cells treated with 3-NP taken to be 100%.

genase (51±2.6% of control) were found to have decreased protein expression SVGA-Tat cells treated with 3-NP compared to controls (Table 2). Typically in neurological diseases, protein expression levels identified by proteomics have changes of one to two fold differences [27]. For example, DRP-2 has about a 50% decrease protein expression level in Down Syndrome and Alzheimer's disease brain [27]. Three proteins exhibited a significant decrease in protein carbonyls in SVGA-Tat cells treated with 3-NP compared with SVGA-pcDNA cells treated with 3-NP: actin (81±2.3% of control), calreticulin precursor protein (79±2.2% of control), and synovial sarcoma X breakpoint 5 isoform A (74±1.6% of control) (Table 3).

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 astrocytes expressing Tat were protected against oxidative insults including 3-NP, while other cell types expressing Tat experienced cell death [11]. In SVGA-Tat cells treated with 3-NP, a mitochondrial toxin, we found heat shock protein 90 and mitochondrial single-stranded DNA binding protein to have increased protein expression levels, while lactate dehydrogenase, and β-actin had decreased protein expression levels compared to controls. Further investigation of oxidized proteins by proteomic analysis identified three proteins, β-actin, calreticulin precursor protein and synovial sarcoma X breakpoint 5 isoform A, to be less oxidized in SVGA-Tat cells treated with 3-NP compared to SVGA-pcDNA cells treated with 3-NP.

HIV-1 and gp120 have been demonstrated to either up- or down-regulate gene expression [14,50,51,52]. Transcripts regulated by HIV-1 in astrocytes coding for alternative splicing, DNA binding, transcription factor, actin binding, calcium binding, endoplasmic reticulum, and many others had changes in gene expression [56] that parallel changes in protein expression observed in this study. 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 [1].

Cells are known to induce heat shock proteins (Hsps) upon injury, including hypoxia ischemia, seizure, heat, epilepsy, trauma, neurodegenerative disorders and metabolic disorders [5,44] and display cytoprotection in many different types of cells. Hsps also can protect cells from the consequences of protein misfolding and induce anti-apoptotic proteins, such as bcl-2 [21]. Heat shock protein 90 (Hsp 90) is mainly involved in the folding of proteins that are involved in signal transduction, including steroid hormone receptors. Recently, Hsp 90 has been shown to form a kinase-specific chaperone complex, Hsp90/Cdc37 [39]. The Hsp90/Cdc37 complex acts to facilitate Cdk9 folding/stabilization and the production of the mature Cdk9/cyclin T1 P-TEFb complex. The Cdk9/cyclin T1 P-TEFb complex, in turn, plays a direct role in aiding/mediating Tat-specific activation of HIV-1 transcription [39]. SVGA-Tat cells treated with 3-NP displayed increased protein expression compared to controls. These data suggest that Tat is capable of upregulating proteins needed for Tat-specific activation of HIV-1 transcription. The upregulation of the protective protein Hsp90 gives insight into how astrocytes expressing Tat may protect themselves from oxidative stress, including that induced by 3-NP.

Mitochondrial single-stranded DNA-binding protein (mtSSB) is required for mitochondrial DNA replication, maintenance, and development [29]. Functional interactions between mitochondrial DNA polymerase (pol)1 and mtSSB from Drosophila embryos enhance greatly the overall activity of pol, by increasing primer recognition and binding, and stimulating the rate of initiation of DNA strands [13]. The HIV-Tat protein accumulates at the mitochondria in the K562-Tat cells cultured under low serum conditions [28]. We found the mitochondrial single-stranded DNA-binding protein to have increased protein expression levels in SVGA-Tat cells treated with 3-NP. This finding is consistent with the notion that Tat may interact with mitochondrial DNA.

Under anaerobic conditions, depletion of oxygen, and high demand for ATP, lactate dehydrogenase catalyzes the oxidation of NADH by pyruvate to yield NAD^+ and lactate. Oxidation of pyruvate via oxidative phosphorylation generates 38 ATP, while oxidation of pyruvate to lactate produces only 2 ATP. In patients with HIV infection, higher serum lactate dehydrogenase (LDH) levels have been associated with the presence of *Pneumocystis carinii* pneumonia (PCP), histoplasmosis, tuberculosis, bacterial pneumonia, toxoplasmosis, and lymphomas [4]. In the case of PCP, higher LDH levels are associated with increased mortality. In a review of 200 HIV-infected patients, those with a high serum LDH level had a significantly greater chance of fulfilling criteria for acquired immunodeficiency syndrome (AIDS) and having a lower CD4+ lymphocyte count [55]. LDH was significantly increased in patients treated with antiviral drugs ddI+d4T (didanosine plus stavudine), whereas LDH levels remained unaltered in patients under AZT+3TC (zidovudine plus lamivudine) treatment. There was a significant association between increased LDH and an increase in lactate [2]. In the current study, we found that after 3-NP treatment of SVGA-Tat cells, LDH protein expression levels were decreased. The decrease in LDH suggests that the mitochondria in SVGA-Tat cells are not injured and are producing enough ATP for the cell. Thus, we hypothesize that SVGA-Tat cells are able to protect

the cell from 3-NP induced toxicity by protecting the mitochondria, thereby allowing mitochondria to produce ample energy for the cell.

Actin is a major component of the cytoskeleton that plays crucial roles in cell shape, motility, division, adhesion and intracellular transportation. The HIV viral protein Nef is capable of binding to actin, and actin is important for Nef's signaling activity. Initiation of Nef's signaling cascade facilitates activated kinases to regulate actin organization [15]. The HIV protein Vpr also increases F-actin polymerization, induces its subsequent reorganization, and increases the expression of the membrane-actin linking protein ezrin [32]. Actin is also a co-factor in key processes of HIV infection. Following membrane fusion and viral core disassembly, the HIV reverse transcription complex rapidly associates with the host cell actin cytoskeleton, a prerequisite for synthesis of proviral DNA [3]. Actin also binds to many other HIV proteins aiding in the translocation in and out of the nucleus, release of virions from infected cells, translation, and motility of HIV proteins [59]. Actin itself is incorporated into HIV virions [41]. Tat causes dramatic actin cytoskeletal rearrangements such as membrane ruffling, peripheral retraction, and subsequent cytoskeletal disassembly [58]. The cytoskeleton is important for virion assembly and reverse transcription is dependent on intact actin network [3,47,48]. The interaction between actin and viral particle formation appears to be mediated by Gag [34]. SVGA-Tat cells treated with 3-NP displayed decreased protein expression levels and decreased oxidation of actin. Decreased oxidation of β-actin would be consistent with the notion of decreased viral association with the cytoskeleton with consequent HIV-induced protection of astrocytes. Potentially, this change in Tat-expressing astrocytes protects remaining actin from oxidation, thereby protecting astrocytes from oxidative stress induced cell death. Moreover, oxidation of brain proteins seems to be associated with loss of function [7,10,18]. The decreased oxidation of actin in 3-NP treated, Tat-expressing astrocytes is consistent with the idea of preservation of actin function, which may contribute to astrocyte survival under oxidative stress conditions in HIVD.

Synovial sarcoma X breakpoint 5 isoform a is a protein involved in specific translocation between chromosomes X and 5 identified in synovial sarcomas. In SVGA-Tat cells, Tat expression is predominant not only in the nucleus, but also in the cytoplasm [11]. In the current study, proteomics analysis revealed that synovial sarcoma X breakpoint 5 isoform a is less oxidized in SVGA-Tat cells treated with 3-NP compared to SVGA-pcDNA cells treated with 3-NP. This finding is consistent with the notion that astrocytes expressing Tat protect DNA from oxidation, thereby protecting the astrocyte while enhancing possible Tat–DNA interactions and viral replication. These findings also are consistent with the fact that many HIV patients develop various cancers. There may exist common pathogenetic pathways in HIV and synovial sarcoma tumors. Additional studies would be necessary to confirm this hypothesis.

Calreticulin is a well-known endoplasmic reticulum (ER) chaperone and ER calcium binding protein that assists with the folding of the low-density lipoprotein receptor-related protein (LRP) [40]. Calreticulin binds N-linked glycosylated proteins and promotes folding, oligomerization, suppression of degradation [16], and regulates the retention of misfolded and not-yet-folded glycoproteins in the ER [17]. Calreticulin assists the folding of gp160 and isolated gp120 ([12,26,42]). When gp160 attains its native conformation it is transported to the Golgi complex, where the protein is cleaved into gp120 and gp41 ([35,49]). The gp120 is released to some extent into the cell culture medium. Gp160 is an HIV envelope glycoprotein consisting of two non-covalently associated subunits, soluble gp120 and the transmembrane domain containing subunit gp41. Gp160 is the single HIV component required for fusion between the surface membranes of the virion and the target cell and is the major target for the host's immune system, with most of the antibody activity in the sera of HIV-1 infected humans directed against gp160 [45]. Gp120 has been associated with oxidative stress and neurotoxicity of the cell [37]. SVGA-Tat cells treated with 3-NP displayed decreased oxidation of calreticulin precursor protein. As noted above, oxidation of brain proteins is known to cause loss of function. By protecting calreticulin precursor protein from oxidation ample calreticulin may be produced for proper folding of HIV envelope proteins, with consequent enhanced survival of the virus and its astrocytic host.

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, and oxidative stress may play a role in HIVD ([11,54]). The proteins identified as differently expressed in SVGA-Tat cells after 3-NP treatment, an oxidative stressor, are involved in metabolism (lactate dehydrogenase), protein folding (calreticulin), transcription (mitochondrial single-stranded DNA binding protein and synovial sarcoma X breakpoint 5 isoform A), axonal structure (β-actin), and cytoprotective measures (Hsp90). These proteins may be critical in understanding how astrocytes infected with HIV protect themselves from death induced by HIV and the HIV protein Tat. Further studies will be needed to investigate the relation among these differentially expressed and decreased oxidized proteins and the role of HIV infected astrocytes in HIV dementia.

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