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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-