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HIV alters neuronal mitochondrial fission/fusion in the brain during HIV-associated neurocognitive disorders

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ABSTRACT

HIV-associated neurocognitive disorders (HAND) still occur in approximately 50% of HIV patients, and therapies to combat HAND progression are urgently needed. HIV proteins are released from infected cells and cause neuronal damage, possibly through mitochondrial abnormalities. Altered mitochondrial fission and fusion is implicated in several neurodegenerative disorders. Here, we hypothesized that mitochondrial fission/fusion may be dysregulated in neurons during HAND. We have identified decreased mitochondrial fission protein (dynamin 1-like; DNM1L) in frontal cortex tissues of HAND donors, along with enlarged and elongated mitochondria localized to the soma of damaged neurons. Similar pathology was observed in the brains of GFAP-gp120 tg mice. In vitro, recombinant gp120 decreased total and active DNM1L levels, reduced the level of Mitotracker staining, and increased extracellular acidification rate (ECAR) in primary neurons. DNM1L knockdown enhanced the effects of gp120 as measured by reduced Mitotracker signal in the treated cells. Interestingly, overexpression of DNM1L increased the level of Mitotracker staining in primary rat neurons and reduced neuroinflammation and neurodegeneration in the GFAP-gp120-tg mice. These data suggest that mitochondrial biogenesis dynamics are shifted towards mitochondrial fusion in brains of HAND patients and this may be due to gp120-induced reduction in DNM1L activity. Promoting mitochondrial fission during HIV infection of the CNS may restore mitochondrial biogenesis and prevent neurodegeneration.

1. Introduction

The number of human immunodeficiency virus (HIV) cases has increased to over 34 million individuals worldwide (Brun-Vezinet and Charpentier, 2013). Combined antiretroviral therapies (cART) have increased HIV-positive patients' life expectancy (Samji et al., 2013); however, HIV-associated neurocognitive disorders (HAND) have become more prevalent or remained at the same levels (Bingham et al., 2011; Clifford and Ances, 2013). HAND severity varies from deficiencies that do not affect daily living, asymptomatic neurocognitive impairment (ANI), to a more severe neurocognitive diagnosis known as HIV Associated Dementia (HAD) (Tozzi et al., 2003). Despite much effort to understand the causes of HAND, therapies targeting the specific mechanisms leading to the neuronal degeneration in HIV-infected brains are unavailable.

Multiple mechanisms of neurotoxicity appear to be at work among individuals with HAND, including HIV activation of apoptotic pathways (Kaul et al., 2001), dysregulation of calcium homeostasis (Lipton, 1994; Nath et al., 2000) and oxidative stress (Nath, 2002; Norman et al., 2008). Altered mitochondrial biogenesis (fission and fusion) is implicated in multiple neurodegenerative disorders, such as Alzheimer's and Parkinson's Disease (Deng et al., 2008; Wang et al., 2009, 2013). Mitochondrial fission is dependent upon the GTPase dynamin-related protein (DRP) 1, and is crucial for regulation of mitophagy as well as general mitochondrial distribution (Taguchi et al., 2007), allowing mitochondria–organelle-contacts, Ca + regulation and ATP supply to distant regions such as axonal and dendritic synapses in neurons (Berthet et al., 2014; Dickey and Strack, 2011; Li et al., 2004). DNM1L activity is

Abbreviations: HAND, HIV-associated neurocognitive disorders; DNM1L, dynamin 1-like; MFN, mitofusin; ECAR, extracellular acidification rate; ANI, asymptomatic neurocognitive impairment; MND, minor neurocognitive disorder; HAD, HIV-associated dementia.

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regulated through nitrosylation and phosphorylation of serines: Ser-616 for activation, and Ser-637 for inactivation (Chang and Blackstone, 2007a, 2007b; Cho et al., 2009; Nakamura et al., 2010; Taguchi et al., 2007; Yan et al., 2015). Mitochondrial fusion requires two GTPase proteins, Mitofusin 1 and 2 (MFN) and optic atrophy (OPA) 1 (Chan, 2006; Chen and Chan, 2005). Mitochondrial fusion may serve as a stress response to protect cells from apoptotic cell death (Breckenridge et al., 2003) and excessive mitophagy (Gomes et al., 2011, 2012). Overactive mitochondrial fission is associated with apoptosis (Breckenridge et al., 2003), whereas mitochondrial fusion can mediate replenishment of mitochondrial proteins, DNA, and metabolic intermediates, and thus quality control (Twig et al., 2008). In healthy cells balance mitochondrial distribution and elongation, and therefore, an imbalance of fusion and/or fission protein activity may lead to dysfunction and cell death (Scorrano, 2013).

We recently reported that HIV-Tat promotes CDK5 localization to the cytoplasm of neurons and causes hyperphosphorylation of tau (Crews et al., 2007, 2011; Fields et al., 2015; Patrick et al., 2011). Interestingly, Duboff et al. showed that overexpression of tau leads to mitochondrial elongation and dysfunction via DNM1L mislocalization (DuBoff et al., 2012). Another study linked mitochondrial fusion and elongation with muscle degeneration that was rescued by MFN knockdown or DNM1L overexpression (Deng et al., 2008). Furthermore, HIV protein gp120 induces mitochondrial membrane permeablization and ultimatley leads to apoptosis (Ferri et al., 2000). Further delineating mechanisms by which HIV proteins cause mitochondrial dysfunction and subsequent neurotoxicity may reveal new opportunities for HAND therapies.

Here, we hypothesized that HIV proteins, released from infected cells of the central nervous system (CNS), interact with bystander neurons and cause alterations in mitochondrial fission/fusion processes directly, or as a result of HIV-protein induced mitochondrial dysfunction. To test this we assayed brain tissues from a well-characterized cohort of HIV + donors for expression of key mitochondrial biogenesis proteins. We complemented these studies with *in vivo* experiments using gp120 tg mice, and in vitro assays using SH-SY5Y neuronal cells and primary rat cortical neurons exposed to the HIV protein gp120. Overall, we observed changes in brain protein levels and mitochondrial morphology that suggest mitochondrial dynamics are shifted towards fusion and consolidation of the organelles in the neuronal soma of HAND donors. *In vitro* and in vivo studies suggest that increasing fission activity restores mitochondrial distribution throughout the neuron and neuronal viability.

2. Materials and methods

2.1. Study population

For the present study, we included a total of 27 HIV + cases categorized by HAND severity (normal, ANI, MND or HAD) (Table 1), from the California Neuro-Acquired immune deficiency syndrome (AIDS) Tissue Network (CNTN) at the University of California, San Diego. Cases had neuromedical and neuropsychological examinations within a median of 12 months before death. Most cases died as a result of acute bronchopneumonia or septicemia. Median postmortem interval was 12 h. Autopsy findings were consistent with AIDS and the associated pathology was most frequently due to systemic cytomegalovirus (CMV), Kaposi sarcoma, and liver disease. Subjects were excluded if they had a history of CNS opportunistic infections or Non-HIV-related developmental, neurologic, psychiatric, or metabolic conditions that might affect CNS functioning (e.g., loss of consciousness exceeding 30 min, psychosis, substance dependence). The diagnosis of HIV-encephalitis (HIVE) was based on the presence of microglial nodules, astrogliosis, HIV p24-positive cells, and myelin pallor. HAND diagnoses were determined from a comprehensive neuropsychological test battery administered according to standardized protocols (Woods et al., 2004).

2.1.1. Cell culture

SH-SY5Y cells (rat neuroblastoma) cultured were cultured at 37 °C and 5% CO_2. SH-SY5Y neuroblastoma cells were utilized here for the neuronal phenotype (Biedler et al., 1978). SH-SY5Y were grown in Dulbecco's Modified Essential Medium (DMEM) with 10% fetal bovine serum (FBS) and then differentiated with retinoic acid (50 μM) for 6 days. Cells were then treated with recombinant HIV proteins from NIH AIDS Reagent Program for 24 h: gp120 (10 or 100 ng/mL; clade E, cat# 2968), nef (10 or 100 ng/ml; cat# 11478), or Tat (10 or 100 ng/ml; cat# 2222). Cells were then isolated and lysed for analysis by Western blot, or infected with lentivirus (LV) then treated with Mitotracker Deep Red, fixed and immunostained.

Primary cultures of cortical neurons were prepared from embryonic day 18 Sprague–Dawley rats. The cerebral cortices were collected and triturated gently (3–4 times) in ice-cold Hibernate E medium (Invitrogen) plus 1× B27 supplement (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin. After the tissue settled, the Hibernate E medium was aspirated, and the tissue was triturated for 1 min in 0.1% trypsin in a Ca^{2+}/Mg^{2+}-free phosphate-buffered saline solution supplemented with glucose (1.5 mm), after which trypsin was inactivated by addition of soybean trypsin inhibitor (0.1 mg/ml). The mixture was transferred into Hibernate E medium containing 20 units/ml DNase (Promega) in 0.2× reaction buffer (Promega), and the cells were centrifuged at 200 ×*g* for 1.5 min. The supernatant was quickly aspirated, and the cells were resuspended in 10 ml of Neurobasal (E) medium (Invitrogen) plus glutamate (0.4 μg/ml), 0.5 mm l-glutamine, penicillin, and streptomycin (100 units/ml and 100 μg/ml, respectively), 1× B27 supplement, and 5 mm sodium pyruvate. Once in suspension, the cells were diluted into 30 ml of the same medium without pyruvate (initial plating medium), and the number of viable cells was determined by trypan blue exclusion. Cells were plated on poly-d-lysine-coated Seahorse XF96 assay plates or on glass coverslips in 12-well plates at a concentration of 25,000 cells per well or 70,000 cells per well, respectively, and kept at 37 °C in a 5% CO_2 incubator. After 4 days *in vitro*, the initial plating medium was diluted with an equal volume of maintenance medium of the same composition lacking glutamate and l-glutamine and supplemented with 1% GlutaMAX-1 (Invitrogen). Cultures were fed every 3–4 days by replacement of one half the conditioned media with fresh maintenance media. All experiments were performed with cultures that were 13–15 days *in vitro*. These cultures were 91–95% neuronal, as estimated by immunocytochemical staining according to the manufacturer's protocols with anti-neuronal nuclei (Chemicon, mAB377) or anti-neurofilament 200 kDa (Calbiochem, IF06L). Cells were then treated with recombinant HIV

Table 1
Demographic (sex and age) and clinical data (brain weight, plasma and csf viral load and CD4 count) for the HAND donor cohorts. Donors were assessed for HAND status and classified as "Normal", asymptomatic neurocognitive impairment (ANI), minor neurocognitive disorder (MND) or HIV-associated dementia (HAD).

Group	n	Gender (M/F)	Age	Brain weight (gr)	Plasma VL log	CSF VL log	CD4
Control (HIV −)	4	3/1	50.0 ± 8.1	1330.0 ± 98.9	N/A	N/A	N/A
Normal	7	6/1	46.7 ± 8.2	1357.5 ± 219.9	4.5 ± 2.11	3.3 ± 2.3	272.6 ± 465.2
ANI	5	4/1	44.5 ± 10.7	1240.0 ± 242.5	4.3 ± 1.4	1.8 ± 0.1	40.0 ± 31.3
MND	6	6/0	45.4 ± 5.6	1319.0 ± 220.1	5.2 ± 1.1	2.6 ± 1.4	40.4 ± 77.2
HAD	9	8/1	45.8 ± 7.6	1218 ± 175.8	4.8 ± 2.1	4.8 ± 0.8	76.6 ± 117.1