Clinical Microbiology 2015: Mechanisms of anti-retroviral drug-induced changes in amyloid precursor protein processing: Implications for HAND- Kelly L Jordan-Sciutto- University of Pennsylvania

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Abstract

HIV-associated neuro-cognitive disorders (HAND) persist in 30-50% of HIV positive patients despite viral control by antiretroviral therapy (ART). Several studies indicate a potential role for anti-retrovirals in the persistence of HAND and evolution from a sub-acute, sub-cortical dementia to a cortical, neurodegenerative disease. Based on their ability to induce ER stress in a wide variety of cell types, we hypothesized that HIV protease inhibitors (PI) induce ER stress in the CNS, resulting in chronic dysregulation of the unfolded protein response (UPR) which in turn alters amyloid precursor protein (APP) processing by inducing the Î²-site APP cleaving enzyme-1 (BACE1). Utilizing in vitro and in vivo models, we demonstrate that PIs induce neuronal ER stress leading to PKR-like ER kinase (PERK)-dependent phosphorylation of the eukaryotic translation initiation factor 2Î± (eIF2Î±), and enhanced translation BACE1. Additionally, we demonstrate enhanced AÎ² production, by the PI, ritonavir, in primary rodent neuroglial cultures and Chinese Hamster Ovary (CHO) cells expressing human APP. Genetic excision of PERK in primary neurons abrogated the ability of PIs to induce the UPR, phosphorylation of eIF2Î± and translational up-regulation of BACE1. Consistent with these findings, ARVs administered to SIV-infected macaques resulted in elevated levels of BACE1 in the CNS coinciding with markers of neuronal damage. Altogether, these findings implicate PIs as potential mediators of neuro-degeneration in HAND.

Key words:

Cell Cycle, Oxidative Stress Response, Antioxidant Response, Endoplasmic Reticulum Stress Response, PERK, Integrated Stress Response, Neurodegeneration, Transcription, Primary Culture, HIV associated Neurocognitive Disorder, HIV encephalitis, Alzheimer Disease, Parkinson disease, Retinoblastoma, E2F1, neuroinflammation.

Description of Research

Our laboratory investigates molecular mechanisms underlying neurodegenerative processes in the hopes of identifying common and unique players in determining neuronal dysfunction and survivall= among several neurodegenerative diseases driven by neuroinflammation. Currently we are focusing our research efforts on the role of cell cycle proteins, the endogenous antioxidant response and unfolded protein response in three neurodegenerative disorders: HIV encephalitis (HIVE), Alzheimer's disease (AD), and Parkinson's disease (PD).

While HIVE, AD, and PD exhibit different pathologic features, theories as to their etiology share common molecular mechanisms including changes in the trophic factor environment, oxidative stress, and activation of CNS inflammatory components. We hypothesize that neuronal response to these neurodegenerative stimuli includes alterations in expression and/or activity of cell cycle proteins. To this end, we and others have shown that key regulators of cell cycle progression, Retinoblastoma susceptibility gene (pRb), E2F1, and/or p53, exhibit altered levels and patterns of expression in HIVE, PD, and AD. These changes are associated with areas of pathology suggesting a role in degenerative processes. In vitro models of neurodegeneration in each of these diseases also exhibit alterations in cell cycle protein subcellular localization. We are using both human tissue and in vitro models to uncover the role of cell cycle proteins, E2F1, MDMx (a p53 and E2F1 regulatory protein), and pRb in interpreting neuroprotective vs neurotoxic stimuli in primary human, rat, and mouse neuroglial cultures stimulated with trophic factors, chemokines, dopamine, free radicals, beta-amyloid, and HIV-infected macrophage supernatant. These studies are aimed at determining how cell cycle proteins regulate neuronal survival in response to varied and conflicting stimuli. In vitro findings are then used to assess potential roles for these proteins in animal models as well as autopsy tissue relevant to each neurodegenerative condition. Our investigation of E2F1 has resulted in the discovery of a role for this protein in activation of a calpain-dependent death pathway which has not been previously described. Interestingly, neurons responding to HIV-infected macrophage supernatants (our in vitro model of neuronal response to inflammatory infiltrate which mediates HIV encephalitis) activate calpain and increase E2F1 protein levels. One of our immediate lines of investigation is testing the hypothesis that E2F1 induces neuronal death in HIV encephalitis via calpain activation, a novel pathway.

A second area of research in our laboratory is the study of the endogenous anti-oxidant response and its failure to prevent accumulation of oxidative damage and neuronal loss in

neurodegenerative disorders. The two proteins of direct interest to the laboratory are Keap1 and Nrf2. Nrf2 is a transcription factor that regulates the expression of the enzymes responsible for the antioxidant response. Normally, Nrf2 is bound in the cytoplasm by the Kelch ECH associated protein 1(Keap1). However, in response to oxidative stress, sulfhydryl groups on Keap1 become oxidized releasing Nrf2 for translocation into the nucleus. We have recently shown that Nrf2 is aberrantly expressed in AD indicating it is not responding to oxidative stress in neurons of affected brain regions. Interestingly, Nrf2 does appear to be responding appropriately in neurons affected in PD. This has led us to hypothesize that the endogenous antioxidant response is aberrant in AD, but insufficient in PD. Our current studies focus on identifying differences in regulation of the endogenous antioxidant response in AD and PD. The goal of these studies is to explore this pathway as a therapeutic target for neurodegenerative conditions. By enhancing the endogenous anti-oxidant response, neuronal toxicity may decrease leading to increased neuronal function in these patients.

A final area of interest on which our other two lines of investigation has converged is the role of the unfolded protein response (UPR). Induction of the unfolded protein response results in activation of Nrf2 and calpain, proteins activated in response to the endogenous antioxidant response and the E2F1 cell cycle protein respectively. This has led to our investigation of the UPR in neurodegenerative conditions. We are currently looking at pathways activated by the UPR in our various models of HIVE, AD, and PD. The key regulators of this response include pancreatic endoplasmic reticulum kinase (PERK), IRE1, and ATF6. We have already identified increased PERK and phosphorylation of PERK substrate eukaryotic initiation factor 2? in AD tissue and an in vitro model of HIVE. This is consistent with findings by Ryu, E. J., et al. (2002, J. Neuroscience 22:10690) indicating a role for UPR in an in vitro PD model. However, our results indicate that Nrf2 a PERK substrate is not activated in AD suggesting the pathway is compromised in AD. Our future investigations are to determine what parts of the pathway are aberrant in disease progression and identify small molecule inhibitors to block chronic UPR pathway activation which is contributing to neuronal dysfunction and loss.

By assessing the interaction of these three convergent pathways in neurons responding to neurodegenerative stimuli such as oxidative damage, misfolded proteins, and inflammation, we hope to gain a greater understanding of the basic mechanisms underlying neuronal damage, dysfunction and loss in neurodegenerative diseases and identify drugable targets for treatment of AD, PD, and HIVE.